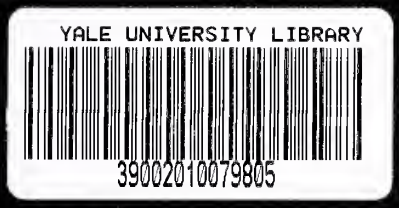


THE ROLE OF CYCLOSPORINE IN LIVER DISEASE AFTER RENAL TRANSPLANTATION

TERRY J. WATNICK

1987

Permission for photocopying or microfilming of

"The Role of Cyclosporine in Liver Disease After Renal Transplantation" for the purpose of individual scholarly consultation or reference is hereby granted by the author. This permission is not to be interpreted as affecting publication of this work, or otherwise placing it in the public domain, and the author reserves all rights of ownership guaranteed under common law protection of unpublished manuscripts.

Terry Watnick

(Signature of author)

TERRY WATNICK

(Printed name)

MARCH 11, 1987

(Date)

The Role of Cyclosporine in Liver Disease After Renal Transplantation

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by

Terry J. Watnick

1987

ABSTRACT

THE ROLE OF CYCLOSPORINE IN LIVER DISEASE AFTER RENAL TRANSPLANTATION

Terry June Watnick

1987

The role of cyclosporine in liver disease after renal transplantation was evaluated. In a retrospective analysis, liver function tests (LFTs) were compared in cyclosporine-treated versus azathioprine-treated renal transplant recipients. In one type of analysis, the incidence and causes of elevated transaminases (defined as SGOT or SGPT greater than 41 IU/L on at least two consecutive occasions) were determined in 19 cyclosporine-treated renal transplant recipients versus 15 azathioprine-treated patients. Forty-seven percent of the cyclosporine group (9 patients) versus 40% (6 patients) of the azathioprine group (p=NS) developed abnormal transaminases during the first 4 to 6 post-transplant months. Peak transaminase levels varied from one and one-half times to ten times normal. Cytomegalovirus (CMV) was the most frequently identifiable cause of hepatic dysfunction (7 of 15 patients).

In another type of analysis, mean monthly SGOT, SGPT and total bilirubin were compared in the two treatment groups. There was no consistent difference in mean SGOT or SGPT between the two groups. Neither SGOT nor SGPT was correlated with serum trough cyclosporine levels. In contrast, total bilirubin levels tended to be higher, although still within the normal range, in cyclosporine-treated

patients versus azathioprine-treated patients. The difference was statistically significant in month 1 (.56 ± .04 vs .42 ± .05, p = .04) and in month 3 (.68 ± .07 vs .44 ± .05, p = .01). Both direct and total bilirubin levels were correlated with cyclosporine trough levels during the first two months when cyclosporine levels were highest.

We conclude that cyclosporine therapy causes hepatic dysfunction characterized by mild hyperbilirubinemia. This effect is most prominent in the early post-transplant period when cyclosporine levels are highest. If mean serum cyclosporine trough levels (measured by high-pressure liquid chromatography) are kept below 200 ng/ml, however, this is of little clinical significance since bilirubin levels remain within the normal range. Because of the many causes of viral hepatitis prevalent during the first six post-transplant months, elevated transaminases alone are not specific for cyclosporine hepatotoxicity. Multiple etiologies must be at least considered before cyclosporine therapy is implicated in a case of hepatic dysfunction.

Acknowledgments

Thank you to:

My sister, Paula, for helping me with the very professional graphs.

Sammy Carr, for typing this manuscript with its many revisions.

Peggy Bia, for teaching me the ins and outs of clinical research and for showing me how much can be accomplished with creativity and determination.

Jodi Halpern, for being my closest friend for the past five years.

KDC, for helping me to maintain my principles in a sometimes hostile environment.

My family, Arthur, Clara and Paula, for listening sympathetically to all my troubles for almost thirty years.

Dedication

To Marc and Ezra - We made it!
Five down and three to go.

TABLE OF CONTENTS

LITERATURE REVIEW

Cyclosporine, a potent immunosuppressive agent, is a cyclic endecapeptide first isolated from two strains of the fungus Tolypocladium inflatum Gams (2,3). Cyclosporine is unique because, unlike other immunosuppressive drugs, it acts directly on the immunoregulatory responses of helper T cells without causing generalized myelosuppression (1).

Cyclosporine is a lipophilic substance that can be given orally or parenterally (3). After oral administration, peak serum levels of the drug are reached within 3 to 4 hours (4). Even with a fixed dosage regimen based on body weight, however, there is a wide variation in blood concentrations of the drug due to erratic absorption by the GI tract (4). Because of its hydrophobic nature, cyclosporine has a large volume of distribution and after chronic administration tends to accumulate in the liver, kidney and fat stores (4,7,8). Thus, with continued use, there is a decreased dosage requirement necessary to maintain constant serum cyclosporine levels (9). In whole blood, in the concentration range from 25 to 500 ng/ml, the uptake of cyclosporine by erythrocytes is about 50% while leukocytes take up 10% to 20% (4). Of the amount of drug remaining in the plasma, 90% is protein bound mainly to lipoproteins (8). The uptake of cyclosporine by erythrocytes is a temperature dependent process and when the temperature is lowered from 37 °C to 21 °C, cyclosporine diffuses into blood cells (4).

Cyclosporine is metabolized in the liver by the cytochrome P 450 oxidase system and drugs interacting with this system can raise or

lower blood levels of the drug (5). Cyclosporine is excreted primarily in the bile while only 6% of the parent drug and its metabolites are excreted in the urine (4). Not all the metabolites of cyclosporine have been well characterized but they are thought to have minimal immunosuppressive properties when compared with the parent compound (5). The toxicity of various metabolites has yet to be determined but the major human metabolite of cyclosporine has no nephrotoxicity in rats (7,12).

Because there is little correlation between dosage and blood concentrations of cyclosporine and because toxicity is thought to be concentration dependent, monitoring of drug levels has become important in the clinical management of patients receiving cyclosporine immunosuppression. There are currently two methods available for this analysis. High-performance liquid chromatography (HPLC) is expensive and labor intensive but is specific for the parent compound (7). The other method, a radioimmunoassay (RIA) using a kit made by Sandoz, is inexpensive and can be used to perform a large number of assays in a relatively short period of time (10). The antibody provided in the kit, however, is nonspecific and detects both the parent compound and some of its metabolites (10). Thus, cyclosporine levels determined by RIA can be 2 to 4 times higher than those measured by HPLC and the difference can vary during the dosage interval (10). In order to minimize this source of variation, most centers monitor cyclosporine trough levels. In general, cyclosporine levels measured by RIA and HPLC parallel each other and either method is adequate as long as the appropriate reference scale is used

(13,14). The one situation in which measurements by RIA may be unreliable is in the setting of liver dysfunction. Burckart et al., reported elevated RIA:HPLC ratios in pediatric recipients of orthotopic liver transplants in conjunction with deteriorating hepatic function, poor bile flow and elevated liver enzymes (15). Since cyclosporine metabolites are excreted in the bile (with little unmetabolized cyclosporine), cholestasis may cause elevated blood levels of these metabolites detected by RIA but not HPLC (15).

For either method, whole blood, serum or plasma may be used. Because cyclosporine can accumulate inside blood cells in a temperature dependent fashion, levels in whole blood are always higher than serum or plasma. The ratio of whole blood cyclosporine levels to serum or plasma is about 2:1 but may vary between patients and according to hematocrit (7,10). Many centers prefer to use whole blood in assaying cyclosporine levels in order to avoid having to equilibrate the sample at a set temperature before processing it. This also makes it easier to compare data between centers. Other investigators, however, prefer to use plasma or serum levels because they are not subject to variation in hematocrit and leukocyte number and may more accurately reflect "free" concentrations (16).

As experience with cyclosporine has been gained, it has become evident that drug levels necessary to achieve immunosuppression without toxicity decrease with time after transplantation (38,39). Data from several centers suggest that serum trough cyclosporine levels (RIA) should be kept under 200 ng/ml especially in long-term treated patients (7,9,14,38,39).

Cyclosporine was first used in renal transplantation by Calne, et al., in 1978 (11). Since then cyclosporine has been shown to be just as, if not more, effective than conventional immunosuppressive therapy. In three large, randomized prospective trials, one year cadaver allograft survival rates in cyclosporine-treated patients were 72% in the European multicentre trial, 80.4% in the Canadian multicenter trial and 84% in the University of Minnesota trial. One-year cadaver graft survival in patients treated with azathioprine and prednisone at these centers was 52%, 64% and 79%, respectively (17,18,19). The difference was statistically significant only in the first two trials. Improved results with azathioprine in the last center were probably due to the addition of other treatment modalities such as antilymphocyte globulin (ALG), pre-transplant splenectomy and multiple transfusions. Similar results with azathioprine have been achieved by other investigators using similar protocols (20,21).

All three groups have followed their patients for at least three years and have demonstrated continued success with cyclosporine (22,23,24).

In addition to improved graft survival, other advantages of cyclosporine in comparison with azathioprine include a decrease in the incidence of acute rejection, a decrease in the incidence of infection, a steroid sparing effect and a decrease in time spent in the hospital (18,19,25,26,37). Also, cyclosporine rarely causes leukopenia (37).

Cyclosporine is not without toxicity. Minor but common side-effects include gingival hyperplasia, hypertrichosis, gastrointestional symptoms, fine tremor and paresthesias (3). Lymphoproliferative disorders, thought to be associated with Epstein-Barr virus (EBV) infection, occur in cyclosporine-treated patients but the risk is no greater than with conventional immunosuppressive therapy (3). Lymphomas seen in the course of treatment with cyclosporine tend to resolve with cessation of therapy (3). The most attention has been focused on the nephrotoxicity of cyclosporine especially in kidney allograft recipients where it can make the diagnosis of rejection difficult. Renal dysfunction caused by cyclosporine can be acute or chronic and generally responds to dosage reduction. Metabolic acidosis, with hyperkalemia and hypertension, may also be results of cyclosporine induced nephrotoxicity (2,3). Although renal allograft recipients treated with cyclosporine consistently have higher serum creatinines than azathioprine-treated patients, renal function does not appear to deteriorate with time (23,25,26,27).

Of all the major side effects of cyclosporine, hepatotoxicity has been the least well characterized. In the initial pilot studies by Calne, et al., in 1978 and 1979, it was noted that almost all patients treated with cyclosporine had abnormalities in liver function consisting of elevated bilirubin, alkaline phosphatase (AP) and in some patients, transaminases as well (11,28). Since then it has been observed that elevated AP in these patients is most often of osseous origin and therefore probably not due to cyclosporine hepatotoxicity (29,44,45).

Subsequent to these initial studies, investigators conducting clinical trials have continued to note elevated liver function tests (LFTs) in renal transplant recipients treated with cyclosporine. The incidence of hepatic dysfunction in these trials has ranged from 3.6% to 42% (2,18,19,30,32,34,35,36). It is unclear whether cyclosporine-induced hepatotoxicity is manifested by elevations in bilirubin alone, transaminases alone or both. Some authors have described hyperbilirubinemia as being characteristic of cyclosporine hepatotoxicity (34,35) but most have also noted accompanying elevations in transaminases (2,18,31,33,36). Differences may be due to a failure in separating those patients with other reasons for hepatic dysfunction. It is generally agreed that cyclosporine-induced hepatotoxicity tends to occur well within the first six post-transplant months (2,31,34,36) and in most cases resolves with dosage reduction rarely necessitating discontinuation of the drug (2,18,30,31,34,36).

The larger, randomized prospective clinical trials described previously have compared liver function in cyclosporine-treated patients and azathioprine-treated patients. Najarian, et al., at the University of Minnesota (19), found that the frequency of elevations in serum bilirubin not due to infectious hepatitis was the same in the cyclosporine group (17% of 121 patients) and in the azathioprine group (15% of 109 patients). Furthermore, between one and one and one-half years post-transplantation, there was no statistically significant difference in serum bilirubin or SGOT between the two groups. These findings are similar to those of the Canadian Multicenter Trial (18) where approximately 4% of each group (103

cyclosporine patients and 107 azathioprine patients) had evidence of hepatic dysfunction. In the cyclosporine group, elevations in LFTs (bilirubin, transaminases and gamma-glutamyl transpeptidase) were associated with cyclosporine trough levels above 1000 ng/ml whereas in the azathioprine group other causes were found (hypoxic liver damage, CMV infection, trimethoprim-sulfamethoxazole toxicity and cholelithiasis). There was no difference in the mean level of bilirubin or aspartate aminotransferase (AST) between the two groups. Cyclosporine-treated patients had a higher mean level of AP but the enzyme was not fractionated. It is thus unclear whether elevated enzyme levels were of hepatic origin.

In the European multicentre trial (17,35), unlike the studies cited above, there was a higher incidence of hyperbilirubinemia in the cyclosporine group (20% of 117 patients) versus the azathroprine group (3% of 115 patients). At one year post-transplantation, cyclosporine-treated patients had higher mean levels of bilirubin, AST and AP than azathioprine-treated patients. Unfortunately, as in other studies, AP was not fractionated. There was no difference in the levels of alanine aminotransferase (ALT) or gamma-glutamyl transpeptidase.

The increased incidence of hyperbilirubinemia in the European trial versus the University of Minnesota trial may in part be due to higher serum levels of cyclosporine in the former. Although serum drug levels were not reported in either study, in the University of Minnesota trial, a lower initial dose of cyclosporine was used and dosages were tapered more rapidly. Other reasons for the disparity

between these trials include possible differences in the frequency with which LFTs were measured and in the methods used to identify patients with causes of hyperbilirubinemia other than cyclosporine. Because these papers did not focus on hepatotoxicity, neither of these parameters was adequately described.

The observation of elevated LFTs in cyclosporine-treated patients has prompted a few centers to examine the hepatotoxicity of cyclosporine in a more systematic fashion. Several factors alluded to above have also made evaluation of these reports difficult. There are many causes of abnormal liver function tests in the immediate post-transplant period, as will be discussed in detail later. In many studies it was unclear how cyclosporine-induced hepatotoxicity was distinguished from other causes of hepatitis. Most authors focused on abnormalities in one enzyme, bilirubin, without looking at transaminases and did not specifically state how often LFTs were measured. This could be important especially in the early post-transplant period when cyclosporine levels tend to be high and episodes of transient hepatotoxicity might be missed. Lastly, because centers used different definitions of hepatotoxicity and different techniques to measure cyclosporine levels, it was difficult to compare results.

One set of reports concentrated on the hyperbilirubinemia characteristic of cyclosporine-induced hepatotoxicity. Laupacis, et al., looked at 21 cadaveric renal transplant recipients treated with an initial cyclosporine dose of 17.5 mg/kg/day further adjusted to achieve serum trough levels (by RIA) of 100-400 ng/ml and two-hour

post dose levels of 400-1000 ng/ml (41). All patients had cyclosporine levels and bilirubin levels measured daily in the hospital. Four patients (19%) had one episode of hyperbilirubinemia (serum bilirubin greater than 1.0 mg/dl for 3 days with no other apparent cause) within two weeks of transplantation lasting 3 to 6 days. A causal relationship was observed between hyperbilirubinemia and cyclosporine levels in these patients. That is, cyclosporine trough levels and 2-hour post-dose levels rose prior to the serum bilirubin and fell, with dose reduction, prior to normalization of bilirubin. The authors conclude that cyclosporine-induced hyperbilirubinemia occurs with trough and 2 hour post-dose cyclosporine levels greater than 400 ng/ml and 1000 ng/ml respectively. In this study, although cyclosporine and bilirubin levels were documented carefully while patients were in the hospital (probably at least two weeks), there is no mention made of how often LFTs were measured after this period and whether or not there were any other LFTs elevated. Also, it is not stated which other causes of hyperbilirubinemia were excluded.

Loertscher, et al., studying only 8 patients receiving cyclosporine (17 mg/Kg/day for 14 days with subsequent monthly reductions of 2 mg/Kg/day) found that 5 patients developed simultaneous increases in bilirubin and cyclosporine within 5 days of transplantation (40). Two additional patients, however, had hyperbilirubinemia without elevations in cyclosporine trough levels later in the post-transplant course. One patient had a high cyclosporine trough level without hyperbilirubinemia. These authors conclude that

hyperbilirubinemia is caused by cyclosporine therapy but is independent of serum drug levels.

Klintmalm, et al., obtained similar results in a larger group of 66 recipients of cadaveric kidneys who recieved 17 mg/Kg/day of cyclosporine for at least 8 weeks post-operatively (42). Eleven of 13 (19.6%) patients who displayed hyperbilirubinemia (bilirubin greater than 2.0 mg/dl) developed it early between 2 weeks and 2 months post-transplant when doses in those patients were still high (mean 17.7 ± 1.1 mg/Kg/day). Three patients had hyperbilirubinemia greater than 6 months post transplant when cyclosporine doses were less than 10 mg/Kg/day. No cyclosporine levels were available but all cases resolved with dosage adjustments. One-half of the patients had normal transaminases (SGOT and SGPT) while in the other half there were elevations to 3 times normal, suggesting that these enzymes are not specific for cyclosporine-induced hepatotoxicity. AP levels in patients with hyperbilirubinemia were normal or slightly above normal. It was mentioned that one of the 13 patients was HBsAg positive prior to transplantation while 7 patients were on other drugs with hepatotoxic potential (cimetidine, isoniazid). None of these drugs was changed during toxic episodes. It was not stated whether other causes of viral hepatitis were investigated.

Another paper by Klintmalm, et al., also suggests that bilirubin is the more important parameter in reflecting hepatotoxicity (39). Of 48 renal transplant recipients treated with cyclosporine (15 mg/Kg/day and then tapered), 18 had isolated increases in ALT while 7 had increases in ALT and bilirubin (ALT greater than 25 umol/L,

bilirubin greater than .70 ukat/L with no other possible explanation). Isolated increases in ALT occurred during the first month in 15 patients and between 2 and 12 months in 3 patients. The mean cyclosporine plasma level (RIA) was not higher in these patients and elevations resolved within 2 weeks without dosage adjustments. Of the 7 patients (14.6%) with increases in ALT and bilirubin, 5 occurred in the first post-transplant month. The mean cyclosporine trough plasma level was higher during these episodes (732 ± 102 ng/ml) than during normal liver function (226 ± 26 ng/ml, $p < .01$). The authors conclude that increases in ALT alone are of little clinical significance while increases in bilirubin and ALT require dosage adjustment for resolution.

The results of Keown, et al., confirm the association between cyclosporine levels and hyperbilirubinemia (9,43). Of 72 cyclosporine-treated patients (dose not stated), 6 patients (8.3%) developed hyperbilirubinemia (bilirubin greater than 1.0 mg/dl) all during the first three post-transplant weeks. Serum cyclosporine levels (by RIA) rose prior to the onset of hyperbilirubinemia, from 65 ± 66 ng/ml to 630 ± 112 ng/ml. All cases resolved after the dosage was decreased. Linear regression analysis showed that serum bilirubin was directly correlated with cyclosporine trough level ($r^2 = .58$, $p = .001$). It was not stated whether other LFTs were elevated as well.

Findings in recipients of other organs also point out the significance of hyperbilirubinemia. Schade, et al., retrospectively studying 30 cyclosporine-treated recipients of heart transplants,

found that serum bilirubin reached a peak elevation, 2.5 times normal at about 2 weeks post-transplant while pre- and post-transplant SGOT, SGPT and AP were not significantly different (48). Interestingly, in 11 subjects, fasting serum bile salt levels were elevated (despite normal AP) indicating pronounced cholestasis. The mean cyclosporine blood level (HPLC) in these patients was 474 ± 47 ng/ml and there was no correlations between drug levels and serum bilirubin or bile salt levels.

Atkinson, et al., retrospectively studying bone marrow transplant recipients, found that 10 of 21 patients had cyclosporine associated hyperbilirubinemia (49). Eight additional patients had other causes for their elevated bilirubin levels, for example, acute graft-versus-host disease of the liver, sepsis and hemolysis. It was not clear that viral hepatitides such as cytomegalovirus (CMV) were ruled out. Cyclosporine-induced hyperbilirubinemia was also associated with an increase in ALT (mean = 109 ± 47 IU/L) but AP levels were only minimally elevated. Cyclosporine trough levels were correlated with bilirubin levels (correlation coefficient = .36). The mean day of onset of hepatotoxicity was 18.5 ± 18 with a mean duration of 72 ± 47.5 days. This prolonged hepatic dysfunction in the face of cyclosporine dose reductions suggests that perhaps patients with viral hepatitis may have been included in this group.

Only the group in Birmingham has made an attempt to compare LFTs in cyclosporine- and azathioprine-treated patients (44,45). In a randomized, prospective study, 35 patients were treated with a cyclosporine dose of 15 mg/Kg/day with reduction to 12 mg/Kg/day at 1

month post-transplant; 31 patients were treated with azathioprine (44). At least some patients had LFTs measured at weekly intervals for the first 12 post-transplant weeks (45). Mean bilirubin levels were significantly higher in the cyclosporine-treated patients for the first 3 post-transplant months but hyperbilirubinemia was uncommon. AST levels were significantly higher in the cyclosporine group only during the first post-transplant month. Mean AP levels were significantly higher in cyclosporine-treated patients at all times but in only one patient was it due to the hapatic isoenzyme. Five patients (14%) in the cyclosporine group and nine patients (29%) in the azathioprine group developed abnormalities in bilirubin or transaminases (bilirubin greater than 22 umol/L, AST greater than 35 IU/L). In the cyclosporine group, elevations were due to CMV (2 patients), herpes simplex (HSV), congestive cardiac failure and possible cyclosporine toxicity although in this patient, LFTs did not normalize with dose reduction. In the azathioprine group the reasons were sepsis (3 patients), viral encephalitis, hepatitis B virus (HBV) infection and 4 cases were unexplained, possibly due to azathioprine or isoniazid. The authors conclude that infection was the most common cause of liver dysfunction following renal transplantation and that cyclosporine has no marked hepatotoxicity. Because bilirubin was higher in the cyclosporine group (although still within the normal range) during the first 3 months, however, it was concluded that cyclosporine is responsible for subclinical hepatic dysfunction. One possible reason for the different conclusions reached by these authors is the frequency with which LFTs were measured.

Although the timing of elevated LFTs was not specifically stated, it is possible that the early hyperbilirubinemia that occurred within the first post-transplant days in other studies was missed. In addition, the initial cyclosporine dose administered by the Birmingham group was lower than in the studies discussed previously.

Only two centers have found elevated transaminases to be indicators of cyclosporine-induced hepatotoxicity. In an abstract, Maddux, et al., reported their study of 46 renal transplant recipients treated with cyclosporine (12-14 mg/Kg/day for 7 to 14 days followed by titration to whole blood trough levels of 400-800 ng/ml (46). Six patients were excluded because of insufficient follow-up, sepsis or viral hepatitis. Of the remaining 40 patients, 16 (40%) had elevated LFTs, mainly SGOT (62%) and SGPT (88%), an average of 41 days post-transplant. Only 1 patient had hyperbilirubinemia. Patients with elevated LFTs had higher whole blood trough levels (920 ng/ml) than patients with normal LFTs (447 ng/ml, $p < .05$) and elevations resolved after dosage reduction. In this study, it is not stated how often LFTs were measured and it is possible that early hyperbilirubinemia was missed. Alternatively, not only were lower doses of cyclosporine used initially, but trough levels were monitored so that hyperbilirubinemia due to early elevations in cyclosporine levels may have been avoided. Patients with sepsis and viral hepatitis were excluded but which types of hepatitis were tested for, i.e., CMV, was unclear. Although elevations in LFTs resolved with dosage reduction, improvement occurred in a mean time

of 60 days. This could easily have been due to resolution of another disease process.

The most comprehensive series reported in the literature is that of Lorber, et al., who followed 466 cyclosporine-treated renal transplants (47). Because of its depth, this study deserves detailed consideration.

Several cyclosporine protocols were used and many patients received a continuous IV cyclosporine infusion (3 mg/Kg) during the first 48 post-transplant hours. Oral cyclosporine at 14 mg/Kg/day was resumed and tapered. After 14 days, the cyclosporine dose was adjusted to maintain serum trough levels of 50-200 ng/ml (RIA). Hepatoxicity was defined as bilirubin greater than or equal to 1.5 mg/dl and/or SGOT or SGPT greater than or equal to 50 IU/L when other potential reasons were excluded. Isolated elevations in AP or LDH were not considered to represent hepatotoxicity. Hepatotoxicity was managed by decreasing the cyclosporine dose to achieve trough levels less than or equal to 100 ng/ml.

Of 466 patients, 228 or 49% had at least one episode of elevated LFTs. Of those patients with hepatic dysfunction, 110 (48%) had hyperbilirubinemia, 108 (47%) had an elevated SGOT and 167 (73%) had an elevated SGPT. Only 1 patient had an isolated abnormality while most (140/228) had elevations in bilirubin or transaminases with increases in AP and LDH. Most patients (187/228) had isolated episodes of hepatotoxicity while 41 patients had recurrent or persistent elevations in LFTs. The mean cyclosporine level was 226 $\pm$ 17 ng/ml in hepatotoxic patients but was not compared to the level in

those patients with normal LFTs. Hepatotoxic patients did have increased bioavailability and decreased cyclosporine clearance when compared with the other patients. In 214 of 228 patients (94%) hepatoxotoxicity began during the initial 90 post-transplant days. In fact, 50 patients exhibited elevated LFTs during the 48 hours of IV infusion and 61% of hepatotoxic episodes began within the first seven days when cyclosporine doses were highest. Dosage reduction resulted in resolution in 81% of the patients including all 14 patients whose elevated LFTs began after 90 days.

The incidence of hepatotoxicity detected in this series exceeds that found by any other group. There are several possible reasons. First, unlike other protocols, patients were treated with continuous infusions of cyclosporine, presumably leading to higher levels of the drug. In fact, almost 1/4 of patients with hepatotoxicity experienced it during this period. Although not explicitly stated, LFTs were probably measured daily in the hospital allowing for increased detection of abnormalities. It would be interesting to know whether elevations occurring during the first post-transplant week were predominantly hyperbilirubinemia, as found in other studies. Lastly, it is unclear from this paper how many patients may have had other causes for abnormal LFTs. For example, of those patients with hepatotoxicity, 16 had a history of polycystic disease, 16 had a history of "hepatitis," 4 had cholelithiasis, 3 had peptic ulcer disease and 3 had pancreatitis. Although it was stated that these problems were corrected prior to transplantation, they could conceivably continue to cause elevated LFTs and additional

investigation might be necessary to rule this out. Furthermore, it would not be at all inconsistent to assume that the 32 patients with recurrent or persistent LFT abnormalities had non A- non B-hepatitis which is the most common cause of hepatitis in renal transplant recipients (51,62). In addition, although the authors state that patients with viral hepatitis were excluded, it might be useful to know which viral infections were tested for in these patients, i.e., CMV, EBV, HBV, HSV.

All of the other studies previously discussed have concluded that cyclosporine-induced hepatotoxicity is without sequelae. Lorber, et al., however, found that 11 (5%) cyclosporine-treated patients with hepatotoxicity developed biliary calculous disease detected between 8 and 33 months post-transplant. Nine of the patients previously had recurrent or persistent cyclosporine "hepatotoxicity." The authors suggest that cyclosporine hepatotoxicity may be linked to biliary calculous disease. No cholelithiasis was seen in 279 azathioprine-treated patients at the same institution. These suggestions must be regarded cautiously since the incidence of biliary calculous disease in cyclosporine-treated patients without hepatotoxicity was not reported. As the authors suggest, longer follow-up of this cohort is necessary.

In sum, the incidence of cyclosporine-induced hepatotoxicity has varied depending on the definition applied and the rigor with which abnormalities have been searched for. Authors that have obtained LFTs frequently in the early post-transplant period when cyclosporine levels vary widely have found a high incidence of hepatotoxicity.

There seems to be general agreement that cyclosporine liver toxicity during this period is manifested by elevations in serum bilirubin often associated with increases in transaminases. These abnormalities which are directly related to blood levels of the drug resolve with dosage reduction. A minority of patients can develop hyperbilirubinemia after this period which is still reversible with dosage reduction. Often, however, serum cyclosporine levels are not absolutely elevated.

Elevations in LFTs after the first few post-transplant weeks have been attributed to cyclosporine but the analysis of many authors has been confounded by a failure to consider the many other causes of post-transplant hepatitis. Post-transplant hepatitis is a common phenomenon and long before the advent of cyclosporine, liver dysfunction was noted to occur in 7 to 67% of renal transplant recipients with 6 to 16% of these patients developing chronic hepatitis (50,51). The significance of these figures cannot be underestimated since in one study the most common cause of mortality in renal transplant recipients with grafts surviving more than 5 years was chronic liver disease (52). Death was usually precipitated by extrahepatic sepsis (52). Conversely, patients exhibiting chronic liver disease were found to have decreased survival when compared to patients without elevated LFTs (51,53). It is important to state that all the studies of liver disease in renal transplant recipients were done prior to the cyclosporine era. The complexities inherent in assigning an etiology to elevations in LFTs after renal

transplantation are best appreciated by a brief consideration of the many possible causes of post-transplant hepatitis.

Although at one time HBV infection was thought to play a major role in post-transplant hepatitis, the importance of this agent has waned due to the introduction of tests to screen banked blood for HBV surface antigen (HBsAg) (51,62,63,66,69). In one large series, for example, Ware, et al., found that HBV accounted for only 10% of liver disease detected in renal transplant recipients (62). HBV is still thought to be an important factor in chronic hepatitis (53,70), however. HAV appears to play no role in the development of post-transplant hepatic dysfunction (51,62).

Clearly, then, post-transplant hepatitis in the majority of cases is due to non A-non B hepatitis. Although many agents have been implicated as the cause of non A-non B hepatitis, in most cases of post-transplant hepatitis it is impossible to find an etiology (51,53,62,63,65,66). This has lead some authors to conclude that the most common cause of hepatitis (especially chronic hepatitis) is transfusion associated, viral, non A-non B hepatitis (51,62).

In the cases where an etiology can be assigned, CMV is the most frequently implicated agent. CMV infection is ubiquitous in renal transplant recipients with active infection rates between 43 and 92% (55). The onset of a large number of CMV infections (as well as CMV hepatitis) occurs within the first 2 1/2 months and almost all occur by the fourth post-transplant month (54,56,57,58,59,61). Primary CMV infection occurs in patients who have no serologic evidence of prior CMV exposure and is thought to be primarily transmitted by an

allograft from a seropositive donor (54,55). Secondary CMV disease, which is more common, happens when the recipient who is seropositive pre-transplant reactivates latent CMV, probably as a result of immunosuppresive medications (54,55,57,60). Active CMV infection is asymptomatic in about two-thirds of cases while only 2%-3% suffer fulminant, disseminated terminal disease (54,56,57,59,61). Those patients with symptomatic CMV can exhibit a syndrome characterized by fever, leukopenia, fatigue, pneumonitis and hepatitis (54,55,56). In several large studies of liver disease in renal transplant recipients, CMV has been implicated in 18 to 30% of cases (51,62, 63). Conversely, about 15% of patients with active CMV infection develop hepatitis (55,58,61). Liver dysfunction is more common in patients with primary CMV infection (55). The degree and extent of liver function abnormalities correlates with the magnitude of CMV titer rises and with the general severity of the disease (56,64). CMV hepatitis has been mostly associated with elevations in transaminases, particulary SGOT (56,61). CMV hepatitis in the renal transplant recipient tends to be a transient, self-limited disease but fulminant cases progressing to hepatic failure and death have been reported (62,63,64,65). Several authors have tried to implicate CMV as a cause of chronic HBsAg negative hepatitis in renal transplant recipients since liver dysfunction due to CMV has been reported to last as long as 20 weeks (56,62,64,65). Although hepatic dysfunction and CMV infection often occur concurrently, as Ware, et al., point out, care must be taken in implicating CMV. Because CMV infection is so common, there is always the possibility that these

events overlapped by chance (62). If, however, the onset of liver disease is accompanied by a characteristic febrile illness with positive cultures and subsequent seroconversion, CMV can be assigned as the cause with some certainty (62,65).

In contrast to CMV, other members of the herpes virus family - EBV, HSV and herpes zoster (HZV) - are thought to play a small role in post-transplant hepatitis with sporadic cases reported in several studies (62,63,65,66). This may in part be due to the fact that these viruses are not tested for on a routine basis. As in the case of CMV, a large number of renal transplant patients are seropositive for these herpes viruses prior to transplantation and often latent virus can be reactivated as evidenced by seroconversion (50,54,61,62, 64,67,68). These viruses also have their onset mainly in the first six post-transplant months (54). In the case of HSV and HZV, infections are usually cutaneous in nature but can disseminate causing fulminant terminal hepatitis (50,54,62,63,66). In the case of EBV, infection is most notable for its association with lymphoproliferative disorders but may also cause a CMV-like syndrome with acute hepatitis (54,67,68). To complicate diagnosis further, there has been a suggestion that other herpetic infections can mimic CMV since in some patients there may be concurrent rises in antibodies to CMV and EBV, HSV or HZV along with the symptoms characteristic of CMV (67,68,74).

In addition to the multiple viral causes of post-transplant hepatitis, renal transplant patients often receive a number of potentially hepatotoxic drugs including alpha-methyldopa, isoniazid,

acetaminophen, furosemide and hydralazine (50). In one study, patients recieved a mean of seven proven potentially hepatotoxic drugs (71). Prior to the advent of cyclosporine, in fact, azathioprine was thought by many to be an important etiologic factor in post-transplant liver disease (65,66,71,72). Azathioprine can cause a dose-related cholestatic picture and many series have reported cases of liver dysfunction which reversed with azathioprine reduction or discontinuation (51,62,65,69). Despite this, the overwhelming consensus is that azathioprine and drugs in general are a minor cause of acute hepatic dysfunction and are of no importance in chronic hepatitis (51,62,63,65,66,69,71,72).

Aside from viruses and drugs, other possible causes of abnormal LFTs will only be mentioned and include congestive cardiac failure, diabetes mellitus, polycystic disease, biliary tract disease and ethanol abuse (50,73,76).

This brief review is sufficient to underscore the fact that multiple etiologies must be at least considered before cyclosporine toxicity can be definitively implicated in a case of abnormal LFTs. This is especially true of the viral hepatitides which tend to occur during the same time period as cyclosporine hepatotoxicity, during the first 4 to 6 post-transplant months. Unfortunately, most of the studies that have examined cyclosporine hepatotoxicity have not detailed which other causes of hepatitis were ruled out, making the results difficult to interpret.

PURPOSE

The purpose of this retrospective study was to examine the role of cyclosporine in liver disease occurring during the crucial first 6 months after renal transplantation. Several important questions were addressed. First, are all cases of liver dysfunction that occur during cyclosporine therapy due to the drug itself? Second, do elevated LFTs occur more fequently with cyclosporine than with azathioprine immunosuppression? Lastly, which LFTs, if any, reflect cyclosporine hepatotoxicity? Since liver disease is an important cause of mortality in long-term survivors of renal transplantation, answers to these questions may help to elucidate whether cyclosporine will aggravate the course of this disease.

MATERIALS AND METHODS

Cyclosporine was introduced at Yale in November of 1983. Up until January, 1985 it was used with steroids only in high-risk renal transplant recipients, i.e., diabetics, recipients greater than 50 years of age, recipients of second and third grafts. Since January, 1985, cyclosporine has been used in all recipients of cadaveric kidney transplants.

Patient Population

Liver function tests (LFTs) and cyclosporine levels (where applicable) were analyzed for all patients (n=56) receiving a renal transplant at Yale-New Haven Hospital between August, 1983 and April, 1985. In order to be included, patients had to have had bimonthly LFTs consisting of SGOT and/or SGPT, for the first two months following transplantation and monthly LFTs thereafter for at least two additional months. Bilirubin levels were also analyzed whenever they were available. AP levels were not examined because fractionation to determine percent bone activity was not routinely performed. Patients were excluded from this study if they had chronic elevations in LFTs due to known liver disease prior to transplantation. For the purposes of this study, patients were followed for a minimum of four months and a maximum of six months.

Patients were assigned to the cyclosporine treatment group or the azathioprine group depending upon which drug was used during the second through sixth post-transplant months. Thus, patients in the

cyclosporine-treated group had to have been started on the drug within one month of transplantation and maintained on it for at least four consecutive months; likewise for the azathroprine-treated group. Four patients were switched from azathioprine to cyclosporine between one and four months post-transplant. These patients could not be included in either group and were excluded from analysis, leaving 52 patients.

Of 25 cyclosporine-treated patients, six were excluded. Two patients had chronically elevated LFTs prior to transplantation - one presumed second to polycystic liver disease and the other thought second to non A-non B hepatitis. Four additional patients had insufficient follow-up (one patient died two months post-transplant, one left treatment AMA and two did not have a sufficient number of LFTs measured.) One patient who was included in the cyclosporine group was also maintained on low-dose azathioprine. There was a final total of 19 cyclosporine-treated patients included for analysis.

Of 27 azathioprine-treated patients, twelve were excluded because there were an insufficient number of LFTs measured during the follow-up period. Four patients had one set of LFTs missing but had good follow-up in subsequent months and were included in the study. Another patient that was included had only one set of LFTs measured in the second post-transplant month, no LFTs in the third month, but had increased LFTs in the fifth month. A total of 15 patients thus comprised the final azathioprine treatment group.

Drug Dosages

Those patients treated with cyclosporine from the day of transplantation received a loading dose of 15 mg/kg orally or .5 mg/kg intravenously on the day of surgery. Thereafter patients received a dose of cyclosporine once or twice per day to approximate a serum trough level of 50-150 ng/ml. Cyclosporine levels were measured daily while the patient was in the hospital and weekly thereafter. Cyclosporine-treated patients were given oral prednisone begun at 2 mg/kg/day and tapered to .25 mg/kg/day over the first month.

Patients in the azathioprine group received 2 mg/kg/day with the dosage adjusted for leukopenia and infection. Patients also received prednisone begun at 4 mg/kg/day tapered to .5 mg/kg/day over the first post-transplant month.

Rejection in all patients was confirmed by renal biopsy and treated with pulse steroids (500 mg solumedrol x 3). If the rejection was steroid resistant, the patient was then treated with two to three weeks of UpJohn Antithymocyte globulin (ATG), 15 mg/kg/day, or Ortho monoclonal antibodies against OKT3 cells for ten days.

Definitions and Data Analysis

Elevated LFTs were defined as an SGOT or SGPT of greater than 41 on two consecutive occasions. Total serum bilirubin levels greater than 1.5 mg/dl were considered abnormal. For each increase in LFTs within the first six post-tranplant months, the patient's hospital

and outpatient records were examined for laboratory data, relevant symptomatology, medications and other pertinent clinical information.

Most increases in LFTs were followed up with serum testing for HBV and anti-EBV antibody levels. In addition, serum CMV antibody titers along with urine and saliva CMV cultures were obtained monthly post-transplant for six months on all patients and more frequently if LFTs were elevated.

A number of criteria were used in assessing the cause of an elevation in LFTs. A rise in LFTs was considered to be due to CMV infection if the rise was associated with or followed by seroconversion or a four-fold rise in CMV antibody titer and/or positive CMV cultures. The infection was considered to be primary if the patient was antibody negative pre-transplant and due to reactivation if the patient had been antibody positive (antibody titer greater than 1:8). If a positive serology and elevated LFTs were associated with fever (temperature elevation above 100 F for at least two days) and/or a depression in WBC (3500 cells/mm^3 for at least two days), this was taken to be further evidence of CMV infection.

An elevation in LFTs was considered to be due to an EBV infection if anti-EBV viral capsid antigen (VCAG) titer was greater than 1:160 and if the anti-EBV early antigen (EAG) titer was greater than 1:20.

A patient was considered to have an acute HBV infection if the increase in LFTs was associated with an HBV screen positive for HBsAg and/or IgM antibodies against HBV core antigen. A diagnosis of non A-non B hepatitis was entertained if the patient had persistent elevations in LFTs, especially SGPT, in the absence of the other

viral causes mentioned above. If the elevation was transient, however, and associated with a clinical syndrome consistent with a viral illness (fever, leukopenia) it was designated as "other viral."

An elevation in LFTs was considered to be due to drug toxicity if it was associated with an elevated drug level and if the LFTs decreased with decreasing drug levels or after the drug was discontinued.

Several other causes of elevated LFTs were considered such as passive liver congestion due to cardiac failure, sepsis, fatty liver associated with diabetes mellitus and ethanol abuse.

In addition to the analyses described above, average monthly SGOT, SGPT and total bilirubin were compared in cyclosporine-treated patients versus azathiprine-treated patients. An attempt was made to correlate cyclosporine trough levels with SGOT, SGPT and bilirubin.

Graft loss was defined as nephrectomy, return to dialysis or death of the patient.

Laboratory Methods

LFTs were measured using the EPOS autoanalyzer. Six patients had transaminases measured on serum samples stored at -20 °C for anywhere from 5 to 22 months after collection. Four patients had one sample and two patients had two samples assayed after storage in this fashion. Three patients belonged to the cyclosporine group and three to the azathioprine group. Serum cyclosporine levels were determined by HPLC. The methods used have been described previously (16).

Serum CMV titers were measured by a complement fixation technique that detects mainly IgG antibody to CMV. An antibody titer above 1:8 was considered positive. Techniques used to process urine and saliva CMV cultures are described elsewhere (75).

A hepatitis B screen consisted of HBsAg titer, anti-HBsAg titer and anti-HBV core titer. If a patient was found to be positive for anti-HBV core antibodies, it was determined whether or not they were IgM in type. These tests were all performed via an Elisa technique using kits from Abbott Laboratories.

Serum antibodies against EBV antigens were measured using indirect immunofluorescence. If a patient had an anti EBV VCAG titer greater than 1:160, antibody against EBV-induced early antigens was measured. A titer greater than 1:20 was indicative of acute EBV infection.

Statistical Analysis

Statistical analysis was carried out using SASS and Clinfo series of programs. Groups were compared using the Student's T-test and Chi square analysis. Linear regression analysis was also employed. All results are expressed as the mean ± SEM. P values less than or equal to .05 were considered statistically significant.

RESULTS

Baseline characteristics of the cyclosporine treatment group and the azathioprine treatment group are compared in Table 1. As expected, due to the initial use of cyclosporine only in high-risk patients receiving cadaveric grafts, cyclosporine-treated patients tended to be older and a larger percentage received cadaveric transplants when compared with azathroprine-treated patients. Six and 12 month graft and patient survival tended to be higher in the azathioprine-treated group but the difference was not statistically significant. Again, this is probably due to the initial use of cyclosporine in high-risk renal transplant recipients. When all patients with liver disease (including two cyclosporine-treated patients with known liver disease prior to transplantation) were considered together, their one year survival was 82% compared with 100% for those transplant recipients without liver disease ($p = .1$). Graft survival was 76% vs. 97%, respectively ($p = .04$). Nineteen cyclosporine-treated patients and 15 azathioprine-treated patients form the basis for the rest of this report.

The frequency of elevated LFTs in each group was similar. Forty-seven percent (9 patients) of the cyclosporine group versus 40% (6 patients) of the azathioprine group ($p = .74$) developed abnormal transaminases during the first 4 to 6 post-transplant months. Peak transaminase levels varied from one and one-half to ten times normal. Although serum bilirubin levels were not obtained regularly, no patient had hyperbilirubinemia in the absence of elevated SGOT and/or SGPT.

Each patient with elevated LFTs had an identifiable cause for their hepatic dysfunction. Graphs of post-transplant transaminases including pertinent clinical and laboratory data for each patient with elevations can be found in appendix A. The conclusions reached from analysis of these graphs are summarized in Tables 2 and 3 and are further discussed below.

Individual Causes of Elevated LFTs

CMV: CMV, both reactivation and primary disease, was the most common identifiable cause of elevated LFTs in this study. Of six azathioprine-treated patients with elevated SGOT and/or SGPT, four had acute CMV hepatitis as did three out of nine cyclosporine-treated patients ($p = .31$). Conversely, of six azathioprine-treated patients with CMV infections, four developed hepatitis as did three of ten cyclosporine-treated patients ($p = .3$).

All four azathioprine-treated patients with CMV hepatitis and two of the three cyclosporine-treated patients were symptomatic with a febrile illness and/or leukopenia. Cyclosporine patient #7 was an outpatient during his episode of elevated transaminases and there were no complaints recorded during any clinic visits. Despite this, the peak in his transaminases was so closely related to seroconversion for CMV that this seems the most likely cause.

Three of the four azathioprine patients (#1,2,4) with CMV hepatitis were being treated for rejection with ATG during or just prior to developing CMV. In none of these patients could use of ATG

be related to elevated LFTs. In patients #1 and #4, peak elevations in LFTs did not occur during ATG treatment. In patient #2, elevations in transaminases did occur only during treatment with ATG. Although ATG can cause a hypersensitivity type reaction (similar to serum sickness) and thus could cause elevated LFTs, patient #2 had no evidence of this. The patient's fever pre-dated and post-dated ATG treatment and was probably due to infection with CMV.

In both groups, CMV hepatitis tended to occur between one and three months post-transplant with elevations in both SGOT and SGPT. SGPT tended to be greater than SGOT and could rise as high as 300 to 400 IU while SGOT generally peaked between 100 to 200 IU. In both groups, elevations in SGPT could persist for up to two to three months.

Cyclosporine-treated patient #9 with symptomatic CMV-hepatitis in the second and third post-transplant months is described in detail elsewhere (77) but deserves further comment here. During the first post-transplant week, she had an elevated SGOT with normal total and direct bilirubin while being treated for chest pain in the Coronary Care Unit. The patient had a long history of congestive heart failure which probably accounted for this initial transient elevation in SGOT. Later in her post-transplant course, she went on to develop an intestinal lymphoma which regressed with discontinuation of cyclosporine (77). Interestingly, the patient was not seropositive for EBV yet molecular hybridization studies showed that the tumor cells contained the EBV genome (77).

Other Viral/Non A-Non B: After CMV, the most common cause of elevated LFTs was a viral illness that could not be identified as CMV or HBV. In azathioprine patient #3 and in cyclosporine patient #11, the elevation was transient while in cyclosporine-treated patients #14 and #15, the hepatic dysfunction was chronic.

The cause of elevated transaminases in patients #3 and #11 was designated as other viral because of the temporal association with fever and leukopenia. EBV infection was not ruled out in either patient. Non A-non B hepatitis could not be definitely ruled out in patient #11 since the elevation in LFTs preceded the symptoms by 13 days. Also, the patient's review of systems was positive for jaundice prior to transplantation.

Cyclosporine-treated patients #14 and #15 had elevations in SGOT and especially SGPT throughout the follow-up period. This pattern is consistent with non A-non B hepatitis. In patient #14 the initial increase in SGOT and SGPT was accompanied by a febrile illness which pre-dated and post-dated ATG therapy for rejection. Initially his WBC was greater than 20,000 cells/mm^3 but he developed leukopenia immediately after ATG therapy ended. There was no evidence of chronically elevated LFTs prior to transplantation. Patient #15 had a post-transplant course complicated by a perforated intestinal diverticula requiring ileocolectomy and ileostomy and then further surgery for intestinal obstruction. Although the patient had evidence of sepsis during part of this time, elevated LFTs preceded these events by many weeks. The patient also developed

steroid-induced diabetes mellitus post-transplant, but again elevated LFTs preceded this.

Sepsis: Azathioprine-treated patient #6 and cyclosporine-treated patient #8 had elevated LFTs associated with terminal medical events. Patient #6 was a 34 year old male who was admitted for rejection of his living-related renal graft during the fifth post-transplant month. During treatment with monoclonal antibodies against OKT3 cells, the patient began a progressive, down-hill course marked by elevated SGOT and SGPT, a spreading cutaneous herpetic infection, a question of an infiltrate on chest x-ray, disseminated intravascular coagulation, and decreasing mental status. Of note, the patient was treated with IV acyclovir for disseminated herpes zoster during the second post-transplant month and previously had an episode of cutaneous herpes simplex. Just prior to his demise, blood cultures were positive for gram negative organisms and CSF was FAMA positive (indicative of herpetic infection). Elevated LFTs in this patient can be attributed to a disseminated herpetic infection with bacterial sepsis.

Patient #8 was a 32 year old man with brittle insulin-dependent diabetes mellitus who was admitted during the third post-transplant month for treatment of rejection and for a left arm abscess. His hospital course was marked by sepsis with disseminated intravascular coagulation and he ultimately died after a hypoglycemic seizure. Relatively small elevations of SGOT with marked hyperbilirubinemia during this time were probably due to sepsis. Earlier solitary

elevations in SGOT were probably as a result of extremely elevated blood sugars (generally greater than 700 mg/%).

Miscellaneous: The remaining two cyclosporine-treated patients had more than one possible cause for elevated LFTs and each will be discussed briefly.

Patient #10, a 47 year old black male with a history of congenital heart disease, had a post-transplant course notable for several mild elevations (generally less than 60) in SGPT. During one elevation the patient presented with ataxia characteristic of dilantin toxicity and an elevated dilantin level. Dilantin-induced hepatitis, however, is usually associated with a hypersensitivity reaction which this patient did not have (78). Also, the patient had persistent elevations in SGPT even after dilantin levels returned to normal. Although the patient did have a four-fold rise in his anti-CMV antibody, the waxing and waning in SGPT is not characteristic of CMV hepatitis seen in the other patients. A hepatitis B screen was not done but the patient was HBV negative pre-transplant and acute hepatitis B infection does not usually present with mild chronic elevations in SGPT (79). This patient also developed steroid induced diabetes mellitus and could have had elevated LFTs on the basis of fatty liver. Chronic elevations in SGPT along with the patient's history of cardiac surgery and probable transfusions, make non A-non B hepatitis the most likely possibility.

Patient #12, a 44 year old male, developed transient elevations in SGOT and SGPT while being treated with ATG in the sixth post-transplant month. The patient had fevers and leukopenia, generally

but not exclusively associated with ATG therapy. Concommitantly, he developed a pulmonary infiltrate, rales, weight gain and pedal edema which seemed to resolve with Lasix. Transiently elevated LFTs in this patient could have been due to cardiac failure but a viral illness cannot be definitely excluded because of the possibility of a febrile illness not caused by ATG. ATG seems to be an unlikely cause of elevated transaminases in this patient since he had no other signs of a hypersensitivity reaction.

Comparison of Mean Monthly LFTs

The average SGOT, SGPT and total bilirubin were calculated for each patient by month and then the means were compared in the cyclosporine group versus the azathioprine group. Due to the small number of patients in each group and the similar frequency of clinical hepatitis in each group, all patients were included. Patients could have anywhere from none to 16 determinations for any given month. As seen in Figures 1A and 1B, there was no significant difference in SGOT or SGPT between cyclosporine and azathioprine groups during any post-transplant month. Total bilirubin (Figure 1C) tended to be higher in the cyclosporine group and the result was statistically significant during post-transplant months one and three. The higher mean total bilirubin for the azathioprine group in month 6 is due to patient #6, described previously, who had a mean bilirubin for that month of 5.46 mg/dl while dying of sepsis. This value was five times higher than the other 5 patients and when this value was excluded, the mean was .79 ± .15 ($p = .24$) compared with

the cyclosporine group. When patients with elevated transaminases were excluded, the same trends were found.

Correlation of LFTs With Cyclosporine Levels

Mean monthly cyclosporine trough levels were calculated for each patient and then averaged. A patient could have any where from none to 30 determinations for any given month. As can be seen in Figure 2, cyclosporine levels tended to decline with time, especially after the first two post-transplant months. The 9 cyclosporine-treated patients with liver dysfunction did not have higher serum trough levels of the drug during any post-transplant month when compared to patients with normal LFTs (Table 4).

In order to detect a dose-dependent effect of cyclosporine, a linear regression analysis was used to correlate LFTs with the cyclosporine trough level measured on the same day. The data for direct and total bilirubin is summarized in Table 5.

When all patients were considered (even those with clinical hepatitis), no correlation was found between cyclosporine trough levels and either SGOT ($r = .04$, $p = .44$), SGPT ($r = .001$, $p = .99$), direct bilirubin ($r = .04$, $p = .60$) or total bilirubin ($r = .06$, $p = .40$), when six months of data was examined. However, a correlation between direct bilirubin or total bilirubin became apparent when the first two post-transplant months were analyzed. This became even more prominant when those patients with elevated transaminases due to other causes were excluded. There was no correlation between cyclosporine levels and either SGOT or SGPT.

DISCUSSION

Prior to the cyclosporine era, chronic liver failure was found to be the most common cause of death in renal transplant recipients with grafts functioning more than 5 years (52). Since cyclosporine therapy has been associated with hepatic dysfunction in some reports, this study was undertaken to examine the role of cyclosporine in post-transplant liver disease.

Because of the retrospective nature of this study, LFTs and particularly bilirubin levels, were not obtained regularly. As a general rule, once patients were discharged from the hospital, LFTs were measured more frequently when patients became symptomatic. Since liver dysfunction can be entirely asymptomatic, the potential for an artificially low incidence of liver disease existed. This was especially true for azathioprine-treated patients who had fewer LFTs measured. We attempted to minimize this detection bias by excluding patients with an insufficient number of LFTs. This resulted in a small sample size which should be kept in mind when considering these results.

The issue of cyclosporine hepatotoxicity was addressed in two ways. In the first part of this study the incidence and causes of elevated transaminases were compared in cyclosporine-treated patients versus azathioprine-treated patients. This type of comparison might have allowed us to detect a subtle effect of cyclosporine in influencing liver disease. Our results show, however, that there is no difference in the incidence of liver dysfunction between the cyclosporine group (47%) and the azathioprine group (40%).

Furthermore, a careful analysis of all available data revealed an identifiable etiology in each case of elevated transaminases. In the cyclosporine group, cyclosporine dosages were not adjusted in response to elevated LFTs and in no case could cyclosporine be implicated as the cause of hepatic dysfunction. Despite small numbers, it appeared that the causes of hepatitis were no different in cyclosporine-treated patients versus azathioprine-treated patients. If both groups are considered together, our results are similar to those of other authors studying the causes of post-transplant hepatitis.

In this series, as in others, HBsAg negative, viral hepatitis was the most common cause of elevated LFTs (51,62,63,65,66). In fact, in no patient could HBV be implicated as the cause of liver dysfunction. Although HAV was seldom tested for, other authors have found that HAV plays no role in post-transplant hepatitis (51,62).

Of 12 patients with viral hepatitis CMV infection was the most commonly identifiable cause (7 patients) of acutely elevated transaminases. CMV hepatitis always occurred within the first three post-transplant months and elevated LFTs could persist for up to three months. These characteristics are similar to those reported previously (51,56,62,63,64,65). The contributing role of other herpes viruses in these patients cannot be ruled out. As pointed out by Marker et al. and Balfour et al., seroconversion for EBV, HSV or HZV often accompanies active CMV infection (67,74). Unfortunately none of these viruses was tested for on a routine basis.

Two additional patients had a viral syndrome characterized by transient elevations in transaminases with leukopenia and/or fever that could not be identified as CMV or HBV. As stated above, serologic tests for viruses such as EBV, HSV or HZV were not routinely obtained, thus these viruses could not be definitively ruled out as etiologic agents. Despite this, most studies have found that herpes viruses (other than CMV) play a minor role in post-transplant liver disease (62,63,64,66). In this study, only one patient developed a fulminant, disseminated herpetic infection with accompanying hepatitis (and bacterial sepsis). This type of course in renal transplant recipients with HSV or HZV has been reported previously (50,54,62,63,66).

In at least two patients, hepatic dysfunction was of a chronic nature and transfusion associated non A-non B hepatitis was the most likely cause. With a longer follow-up period, this diagnosis might have been possible in other patients as well, especially since other studies have implicated non A-non B hepatitis as the most frequent cause of chronic liver dysfunction after renal transplantation (51,53,62,66).

In addition to cyclosporine and for that matter azathioprine, patients in this study were on a variety of drugs with hepatotoxic potential including ATG, dilantin, hydralazine, alpha-methyldopa and furosemide. Drug dosages were not adjusted in response to LFTs and in most patients this did not affect the course of hepatic dysfunction. One patient (#10) did present with ataxia due to dilantin toxicity and shortly thereafter with elevated transaminases

as well. Nevertheless, dilantin was probably not the cause of hepatic dysfunction in this patient. He had been on dilantin for many years without any history of liver dysfunction and his transaminases were elevated even after dilantin levels returned to normal. Also, dilantin hepatotoxicity is usually due to a hypersensitivity reaction which this patient did not have. In addition, several patients were treated with ATG during periods of abnormal liver function. In all but one of these patients (#12) a viral cause (CMV) of hepatitis was identified. ATG can cause elevated LFTs on the basis of a hypersensitivity reaction akin to serum sickness. None of these patients, however, had any other signs of this type of reaction. Our results are thus in agreement with those of other published reports showing that medications play only a very minor role in liver disease after renal transplantation (51,62,63,65,66,69).

In sum, cyclosporine does not appear to alter the spectrum of liver disease in patients receiving renal allografts. The type of analysis described above highlights the complexity involved in assigning an etiology to post-transplant liver dysfunction.

Cyclosporine hepatotoxicity was examined in another type of analysis. Mean monthly LFTs were compared in cyclosporine- versus azathioprine-treated patients and an attempt was made to correlate serum cyclosporine trough levels with LFTs. Cyclosporine levels were measured using the HPLC method which is thought to be more reliable in the setting of liver dysfunction (15). All determinations were performed on serum which may more accurately reflect free drug levels (16).

Not surprisingly, no association was found between transaminase levels and cyclosporine therapy. The 9 cyclosporine-treated patients with elevated LFTs did not have higher cyclosporine levels during any post-transplant month when compared with those patients having normal LFTs. Nor was there any consistent difference in mean monthly SGOT or SGPT in the cyclosporine group versus the azathioprine group. Finally, serum cyclosporine levels could not be correlated with SGOT or SGPT using a linear regression analysis even when the 9 patients with other causes for elevated transaminases were excluded.

Although clinical hyperbilirubinemia in the absence of elevated transaminases did not occur, there was evidence of subclinical hepatic dysfunction in cyclosporine-treated patients. Total serum bilirubin levels were higher (though still within the normal range) in cyclosporine-treated patients versus azathioprine-treated patients during the early post-transplant months. The difference was statistically significant during months 1 and 3. Furthermore, cyclosporine levels were correlated with both direct bilirubin and total bilirubin during the first two post-transplant months when cyclosporine trough levels were highest. The correlation became stronger when the 9 patients with other causes for elevated LFTs were excluded.

Although the correlation of direct bilirubin with cyclosporine levels is suggestive of decreased bile flow, it is important to distinguish between isolated hyperbilirubinemia and true cholestasis. In this study the specific tests needed to clarify this point - AP (liver isoenzyme), 5'-nucleotidase, gammaglutamyl transpeptidase

or serum bile acid levels - were not obtained and further work is needed in this regard. The reports of several authors, however, have addressed this issue. Rotolo et al., studying isolated, perfused rat livers, found that cyclosporine in doses of 2 mg/kg and 20 mg/kg decreased bile flow and bile acid secretion (80). Schade et al. prospectively measured fasting bile salt levels in 11 cyclosporine-treated heart transplant recipients and found them to be markedly elevated despite normal AP levels (48). Finally, Lorber et al. noted an increased incidence of cholelithiasis in cyclosporine-treated patients compared with azathioprine-treated patients (47). From these data it seems that hyperbilirubinemia in cyclosporine-treated patients is probably indicative of true cholestasis.

Our findings with regard to hyperbilirubinemia are similar to those of other authors in that a relationship between cyclosporine blood levels and serum bilirubin levels was detected in the early post-transplant period (9,39,43, 49). The magnitude of hyperbilirubinemia, however, was not the same. For example, Laupacis, et al., Klintmalm, et al., and Keown, et al., all found that between 8.3% and 19.6% of their cyclosporine-treated patients developed overt hyperbilirubinemia usually within the first post-transplant month (9,39,41,42,43). There are several possible explanations as to why overt hyperbilirubinemia was not detected in this study. First, most of the hepatotoxic effects of cyclosporine have been observed early. Lorber, et al., reported that 61% of such episodes began during the first post-transplant week (47). Since bilirubin levels were not measured regularly, it is possible that this effect was missed.

Alternatively, cyclosporine levels at this institution were kept quite low. Even during the first post-transplant month the mean cyclosporine level was only 121 ± 15 ng/ml. Although it is difficult to compare because other centers used the RIA technique, serum cyclosporine trough levels in other studies were generally kept between 100 and 400 ng/ml with levels rising above 600 ng/ml during hepatotoxic episodes (39,41,43). Hyperbilirubinemia may have been avoided at this center by keeping cyclosporine levels low. Further support for this theory comes from the University of Minnesota where cyclosporine levels were kept between 100 and 200 ng/ml (HPLC). These authors found no increase in the incidence of hyperbilirubinemia in cyclosporine-treated patients versus azathioprine-treated patients (19).

Despite these differences, findings from this study and others suggest that serum bilirubin levels (and not transaminases) are the more important parameter in detecting cyclosporine hepatotoxicity (39,42,44,45,48). Only Maddux, et al., and Lorber, et al. have reported that cyclosporine immunosuppressive therapy is associated with elevated transaminases (46,47). In the two types of analyses described above we could find no evidence for this association. In this study, every instance of elevated transaminases was analyzed in an depth manner with a review of all clinical and viral data available. In every case an etiology other than cyclosporine was identified. Furthermore, there was no correlation between serum cyclosporine levels and transaminases. McMaster, et al., who undertook a similar type of analysis, obtained the same results. Not

only was there was no difference in the incidence of elevated LFTs in cyclosporine-treated patients versus azathioprine-treated patients, but a careful review revealed that, as in the series reported here, infection was the most common cause of liver dysfunction in both groups. Both Maddux et al., and Lorber et al. do state that patients with other causes of hepatitis were excluded. Given the complexity of this issue, however, the details of this process were not adequately described. For example, Lorber et al. report that 49% of 466 patients developed cyclosporine hepatotoxicity. With the ubiquity of CMV it would be surprising if CMV infection were absent in this large a proportion of renal transplant recipients. Additional information would be essential in deciding whether all cases of elevated LFTs in this study were in fact due to cyclosporine.

CONCLUSION

In sum, cyclosporine appears to play a minor role in the genesis of liver disease after renal transplantation. Neither the frequency nor the causes of elevated transaminases differs between cyclosporine-treated patients and azathioprine-treated patients. Mean levels of SGOT and SGPT are not significantly higher in cyclosporine-treated patients and there is no correlation between either enzyme and cyclosporine trough levels. Elevated transaminases do not appear to be a specific marker for cyclosporine hepatotoxicity but rather due to viral hepatitis which is prevalent after renal transplantation.

The fact that bilirubin levels are higher (though still within the normal range) in cyclosporine-treated patients versus azathioprine-treated patients during the first few post-transplant months does, however, suggest that the drug can cause mild cholestatic hepatic dysfunction. This is further supported by a correlation between cyclosporine trough levels and direct and total bilirubin levels. It is possible that when mean serum cyclosporine trough levels are kept well under 200 ng/ml, as in this study, this is of little clinical significance since bilirubin levels remain within the normal range.

The advent of cyclosporine has not changed the characteristics of post-transplant liver disease that existed in the azathioprine era. Viral hepatitis is still the most common cause of abnormal liver function in renal transplant recipients treated with either cyclosporine or azathioprine.

TABLES AND FIGURES

TABLE 1

BASELINE CHARACTERISTICS

	Cyclosporine	Azathioprine	P Value
Total Number of Patients	19	15	
Mean Age (years)	47±2	35±2	.0008
% with Diabetes	26	13	.43
% Cadaveric Transplant	100	47	< .0004
% First Renal Transplant	84	87	1.00
6 Month Graft Survival (%)	89	100	.49
6 Month Patient Survival (%)	89	100	.49
12 Month Graft Survival (%)	84	93	.61
12 Month Patient Survival (%)	89	93	1.00

TABLE 2

ETIOLOGY OF ELEVATED LFTs IN TRANSPLANT RECIPIENTS TREATED WITH AZATHIOPRINE

Patient	CMV	EBV	HBV	Drugs	Heart Failure	Diabetes	Symptoms	Conclusion	Month of Onset
1	+	-	-	ATG	-	-	febrile illness leukopenia	reactivation CMV	3
2	+	no data	- HAV-	ATG	-	-	febrile illness leukopenia	reactivation CMV	1
3	-	no data	-	-	-	-	febrile illness leukopenia	other viral	5
4	+	no data	-	ATG	-	-	febrile illness leukopenia	primary CMV	1
5	+	-	-	-	-	-	febrile illness	primary CMV	1
6	-	-	-	-	-	-	febrile illness DIC, sepsis cutaneous infection with FAMA (+) CSF blood cultures positive	disseminated herpetic infection with sepsis	6

TABLE 3

ETIOLOGY OF ELEVATED LFTs IN TRANSPLANT RECIPIENTS TREATED WITH CLCLOSPORINE

Patient	CMV	EBV	HBV	Drugs	Heart Failure	Diabetes	Symptoms	Conclusion	Month of Onset
7	+	-	-	-	-	-	None	primary CMV	2
8	-	no data	-	-	-	+	sepsis, DIC left arm abscess	sepsis	4
9	+	-	-	-	+	-	hepatomegaly febrile illness	reactivation CMV congestive heart failure	1
10	+	no data	-	Dilantin	-	+	-	reactivation CMV vs. non A-non B hepatitis vs. fatty liver vs. Dilantin	3
11	-	no data	-	-	-	-	febrile illness	non A-non B hepatitis vs. other viral	2
12	-	no data	-	ATG	+	-	febrile illness, leukopenia	other viral vs. congestive heart failure vs. ATG reaction	6
13	+	-	-	-	-	-	febrile illness, leukopenia	primary CMV	3
14	-	-	-	ATG	-	-	febrile illness, leukopenia	non A-non B	1
15	-	no data	-	-	-	+	sepsis, bowel perforation	non A-non B	1

TABLE 4

MEAN MONTHLY CYCLOSPORINE LEVELS IN
PATIENTS WITH ELEVATED VS. NORMAL TRANSAMINASES

Post-transplant Month	Elevated Transaminases (n = # of patients)	Normal Transaminases (n = # of patients)	P Value
1	108 ± 22 (9)	133 ± 19 (10)	.41
2	114 ± 30 (9)	97 ± 12 (10)	.60
3	78 ± 9 (9)	72 ± 8 (10)	.59
4	53 ± 6 (9)	62 ± 11 (10)	.50
5	77 ± 14 (6)	84 ± 14 (10)	.74
6	75 ± 11 (4)	88 ± 21 (8)	.68

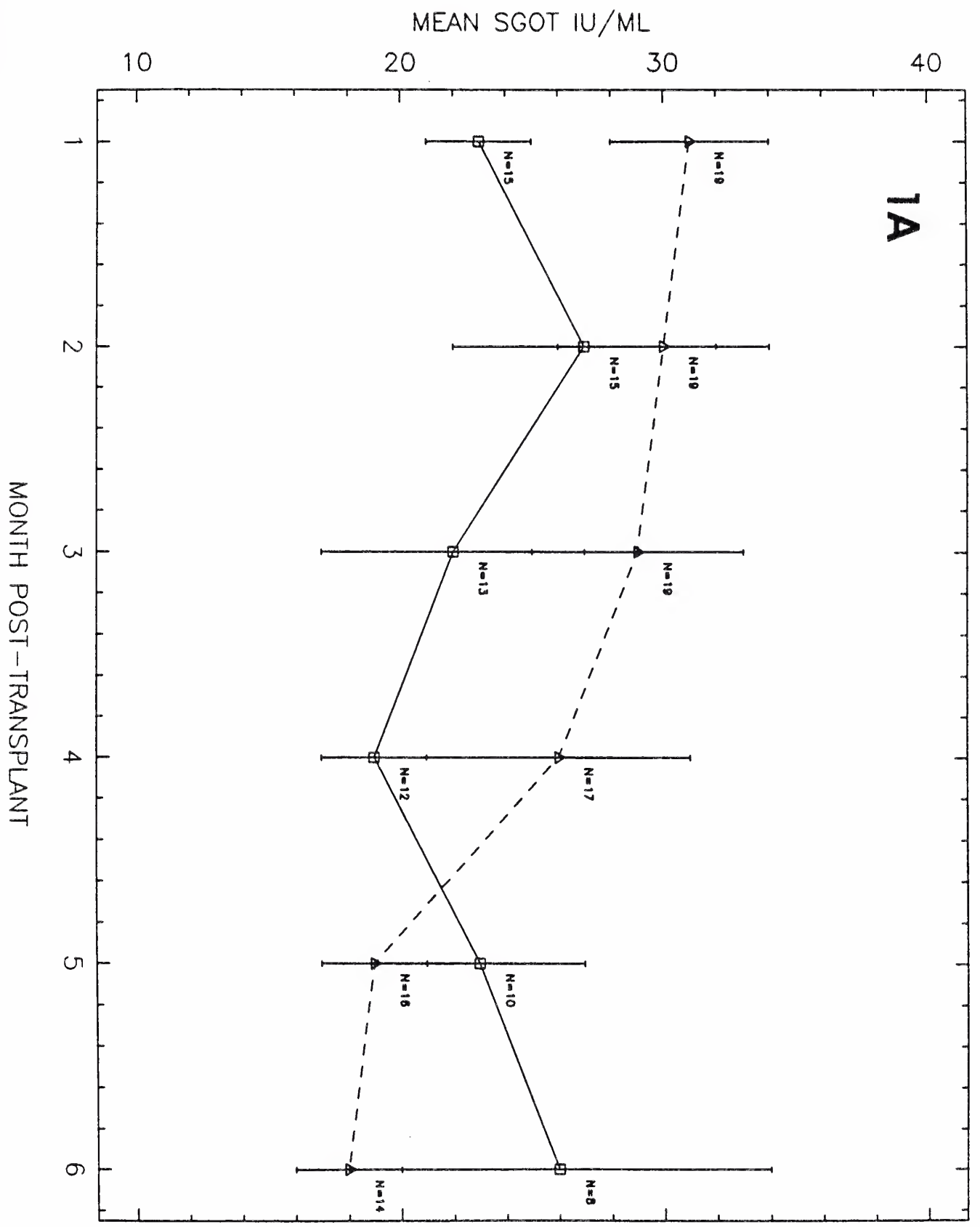
TABLE 5

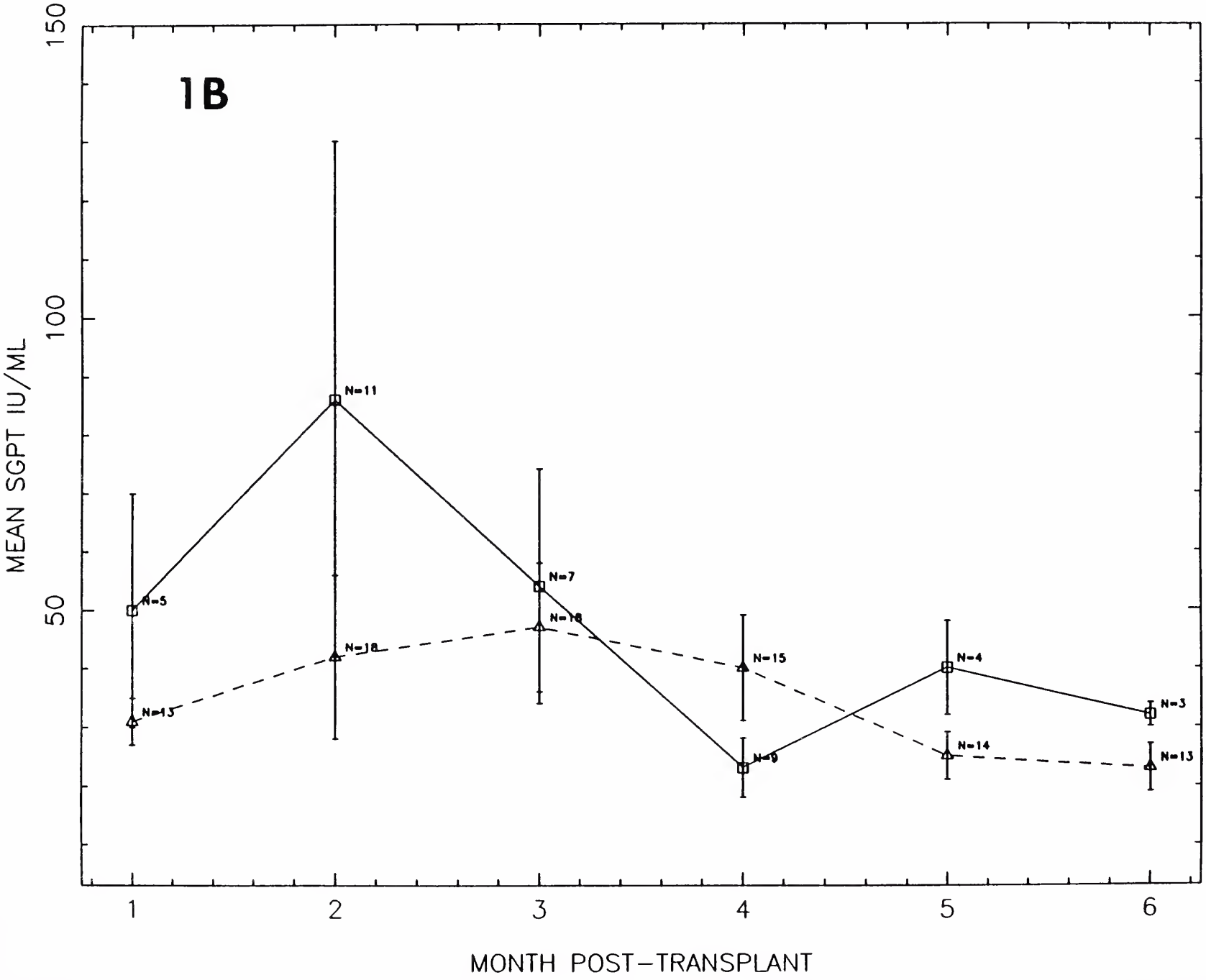
CORRELATION OF CYCLOSPORINE LEVELS WITH
DIRECT AND TOTAL BILIRUBIN LEVELS

	Direct Bilirubin	Total Bilirubin
All patients (2 months of data)	$r = .40$, $p < .001$	$r = .30$, $p = .001$
All patients (6 months of data)	$r = .04$, p .60	$r = .06$, $p = .40$
Patients with normal transaminases (2 months of data)	$r = .74$, $p < .001$	$r = .41$, $p = .003$
Patients with normal transaminases (6 months of data)	$r = .64$, $p = .0001$	$r = .34$, $p = .008$

Figure 1: Mean Monthly LFTs in Cyclosporine-Treated Patients Vs Azathioprine-Treated Patients.

An average monthly SGOT (A), SGPT (B) or total bilirubin (C) level was calculated for each patient (N). There were anywhere from 2 to 16 monthly determinations for each patient. The mean value + SEM for each month was compared in the cyclosporine group (Δ --- Δ) versus the azathioprine group (□——□): "<": P < .05


MEAN SGOT IU/ML
10
20
30
40
1
2
3
4
5
6
MONTH POST-TRANSPLANT
1A
N=15
N=19
N=15
N=19
N=13
N=19
N=12
N=17
N=16
N=10
N=14
N=8


1B
150
100
50
MEAN SGPT IU/ML
1
2
3
4
5
6
MONTH POST-TRANSPLANT
N=5
N=11
N=7
N=9
N=4
N=3
N=15
N=18
N=18
N=15
N=14
N=13

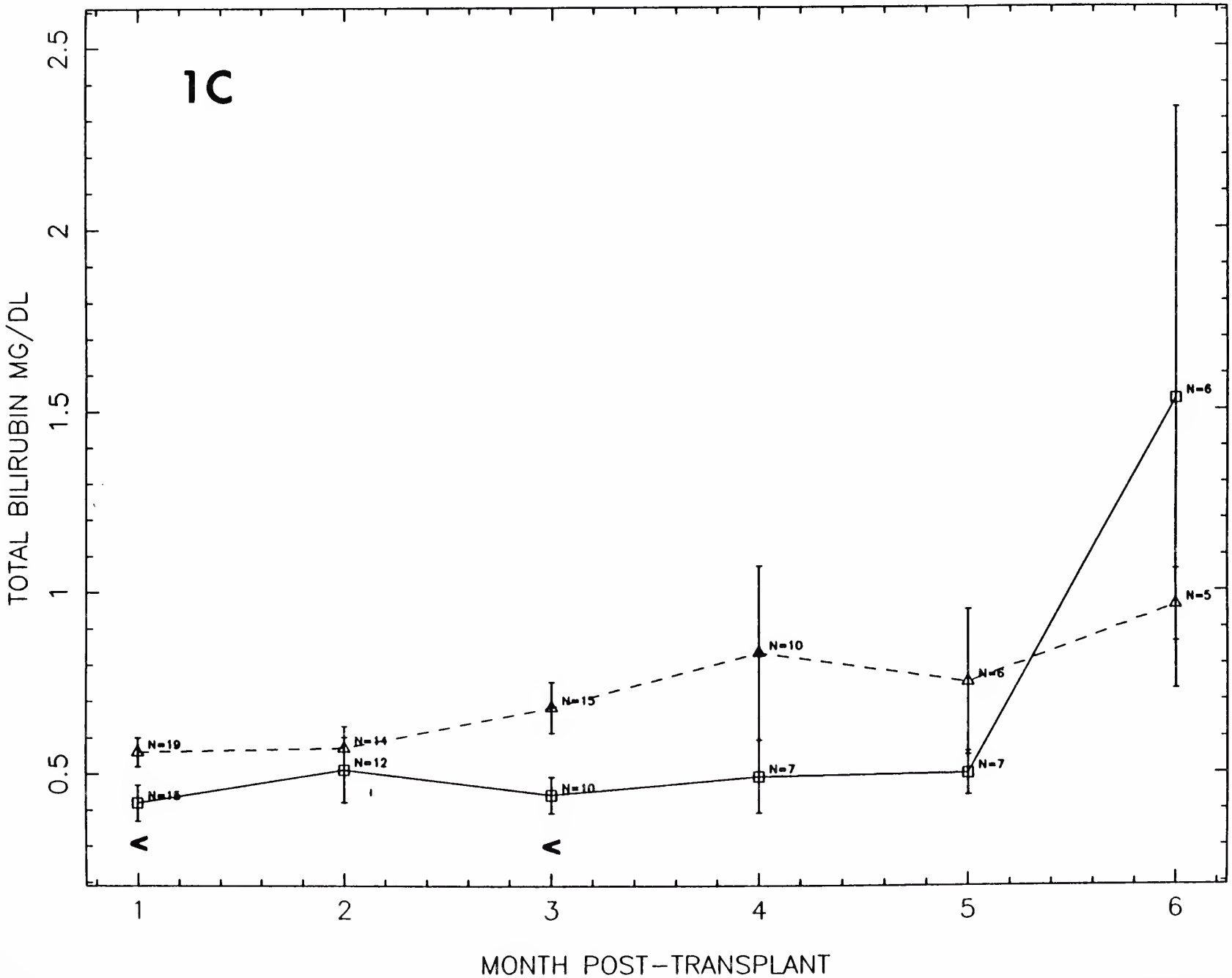
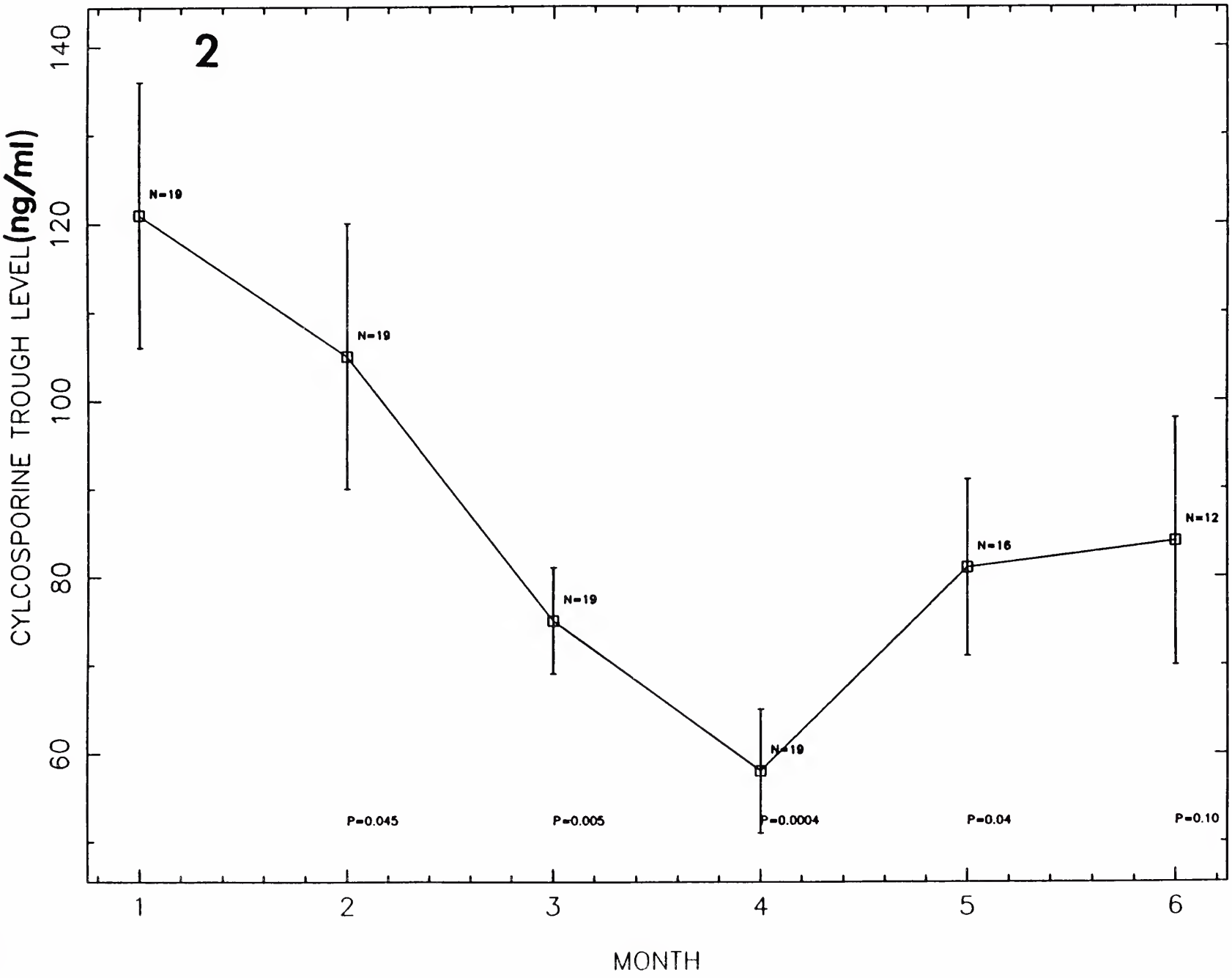

1C
TOTAL BILIRUBIN MG/DL
0.5
1
1.5
2
2.5
1
2
3
4
5
6
MONTH POST-TRANSPLANT
N=19
N=14
N=15
N=10
N=6
N=5
N=16
N=12
N=10
N=7
N=7
N=6

Figure 2: Mean Monthly Cyclosporine Trough Levels.

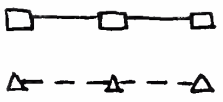
Cyclosporine trough levels were measured on serum using High-Performance Liquid Chromatography. An average monthly cyclosporine trough level was calcualted for each patient (N). There were anywhere from 2 to 30 monthly determinations for each patient. Mean ± SEM cyclosporine trough levels during post-transplant months 2, 3, 4, 5 and 6 were compared with the level during month 1.

MEAN MONTHLY CYCLOSPORINE TROUGH LEVELS



APPENDIX: Clinical Data for Nine Cyclosporine-Treated Patients and Six Azathioprine-Treated Patients with Elevated Transaminases.

LEGEND:



: SGOT

: SGPT

CMV: Cytomegalovirus Serology

HBV: Hepatitis B Virus Serology

EBV: Epstein-Barr Virus Serology

CMV CULT: Cytomegalovirus Cultures

U: Urine

S: Saliva

Pre-TPX: Pretransplant

MCA: Ortho monoclonal antibodies

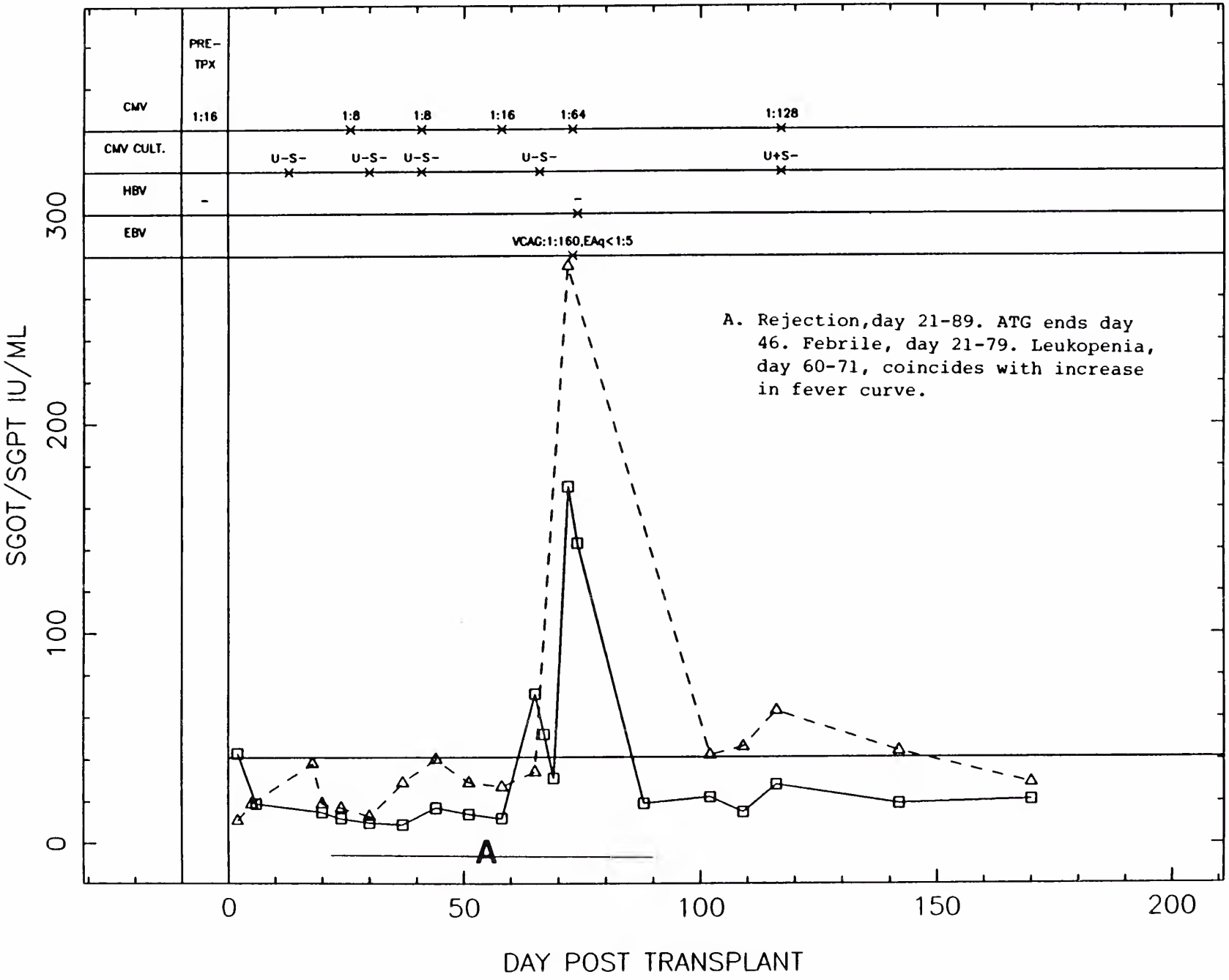
RLL: Right lower lobe

L: Left

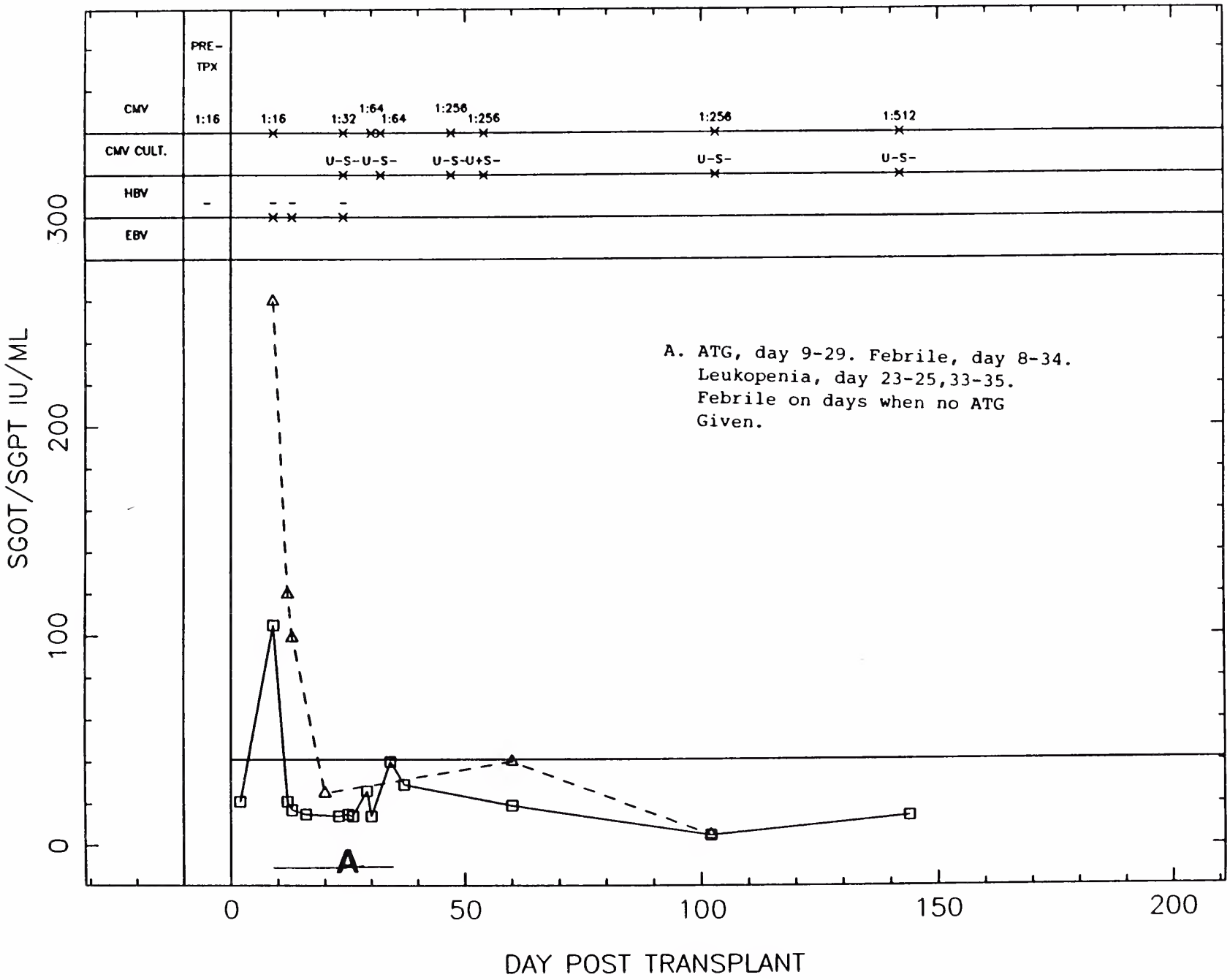
Wgt: Weight

Cxr: Chest x-ray

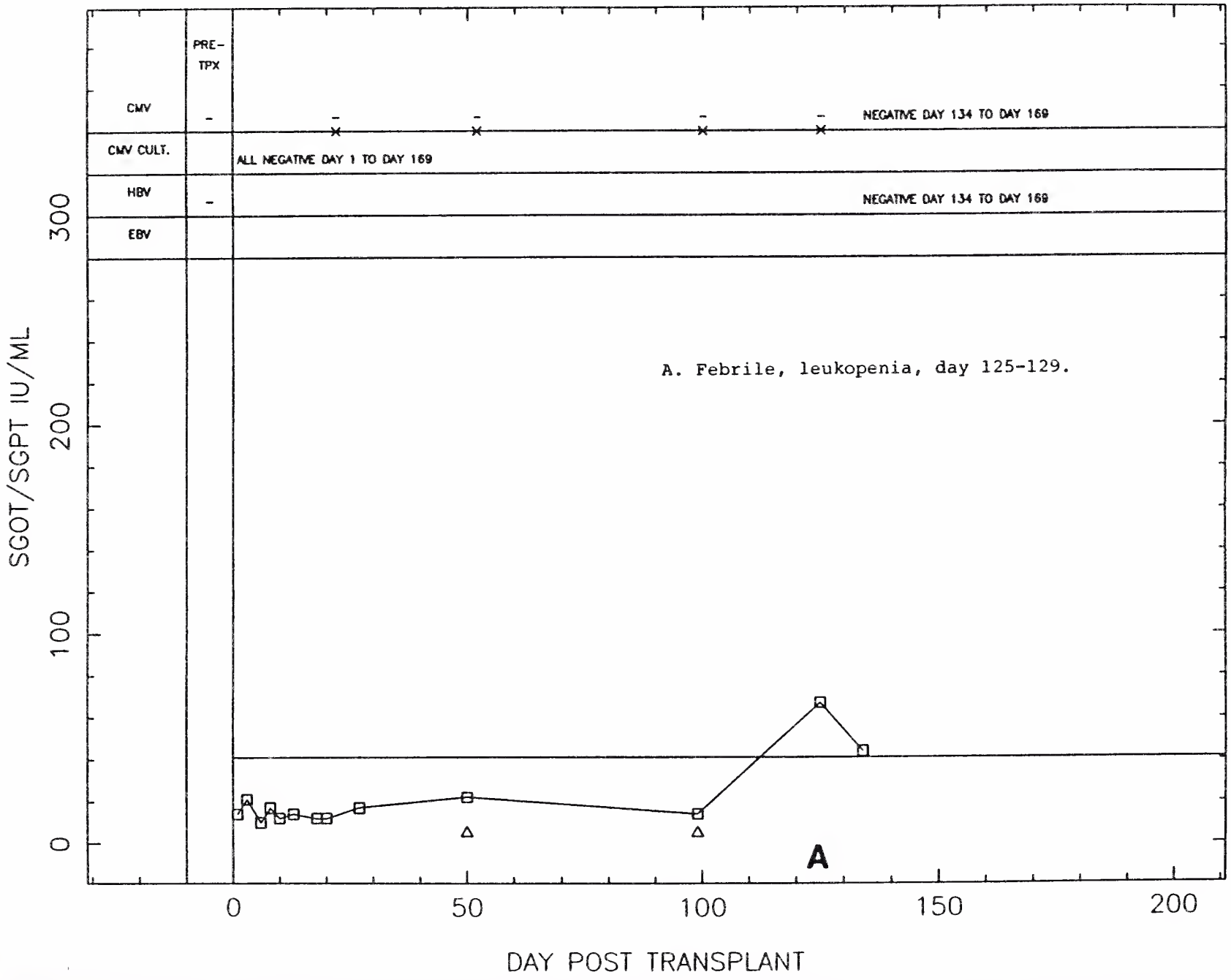
AZATHIOPRINE:PATIENT NUMBER ONE



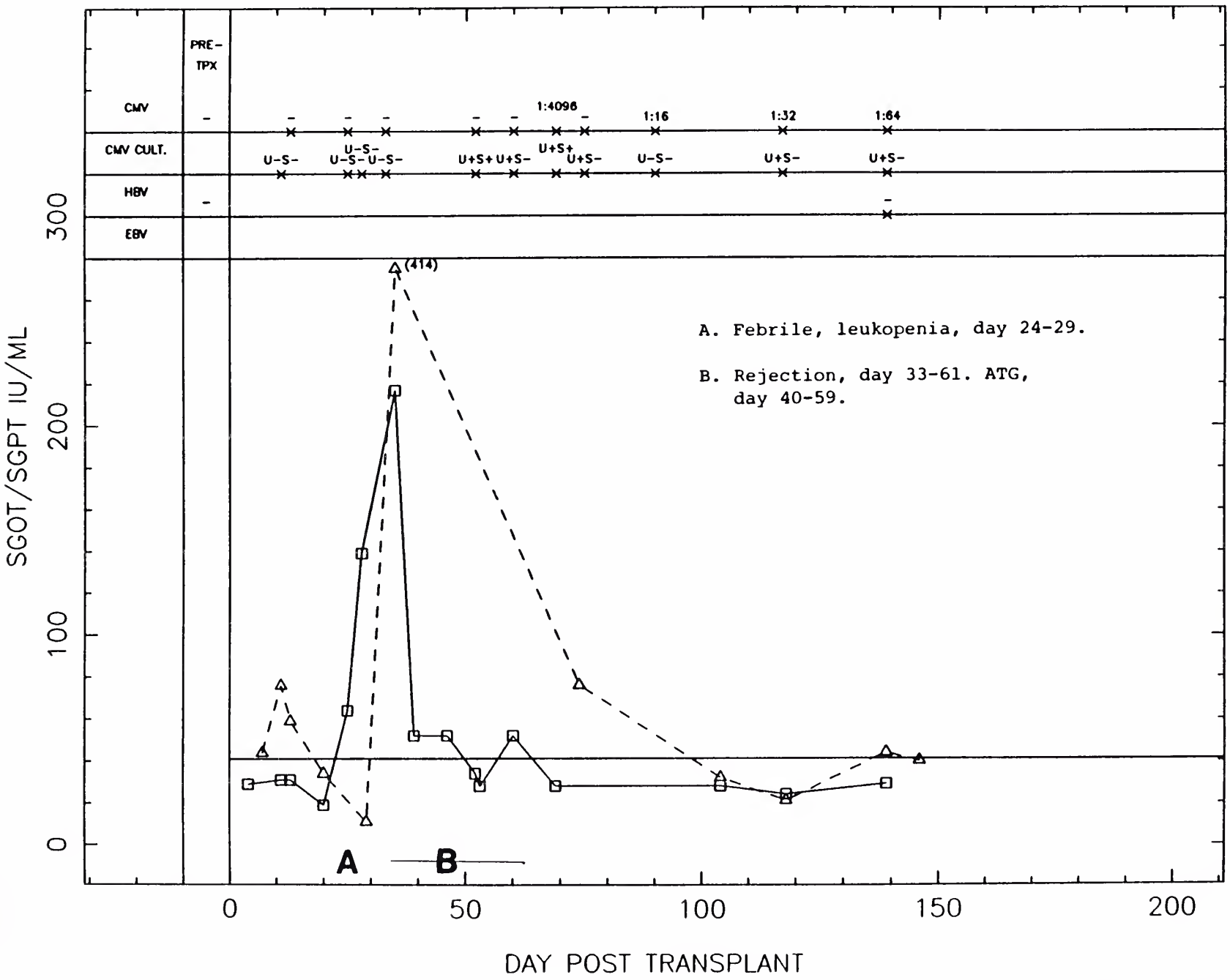
AZATHIOPRINE:PATIENT NUMBER TWO



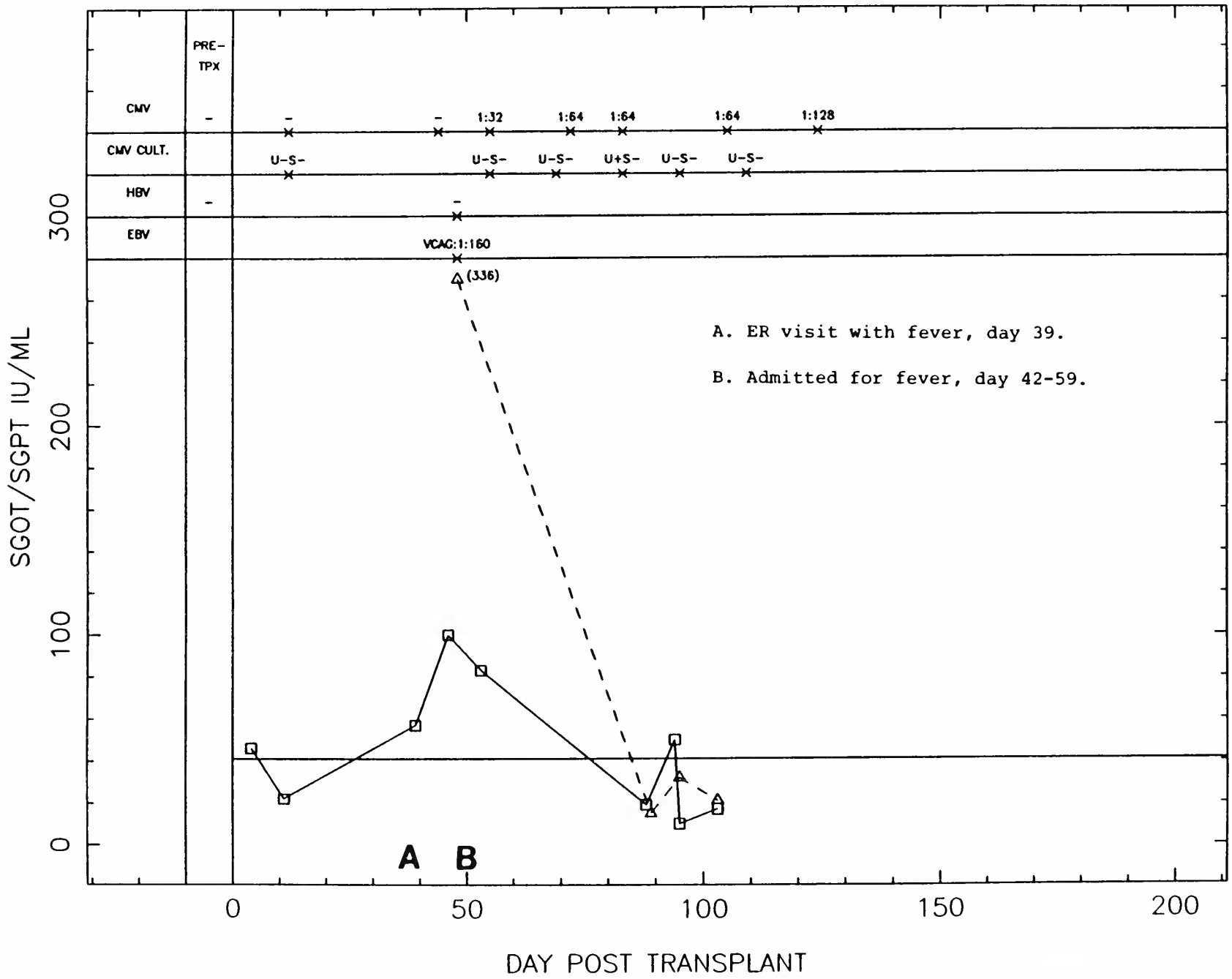
AZATHIOPRINE:PATIENT NUMBER THREE



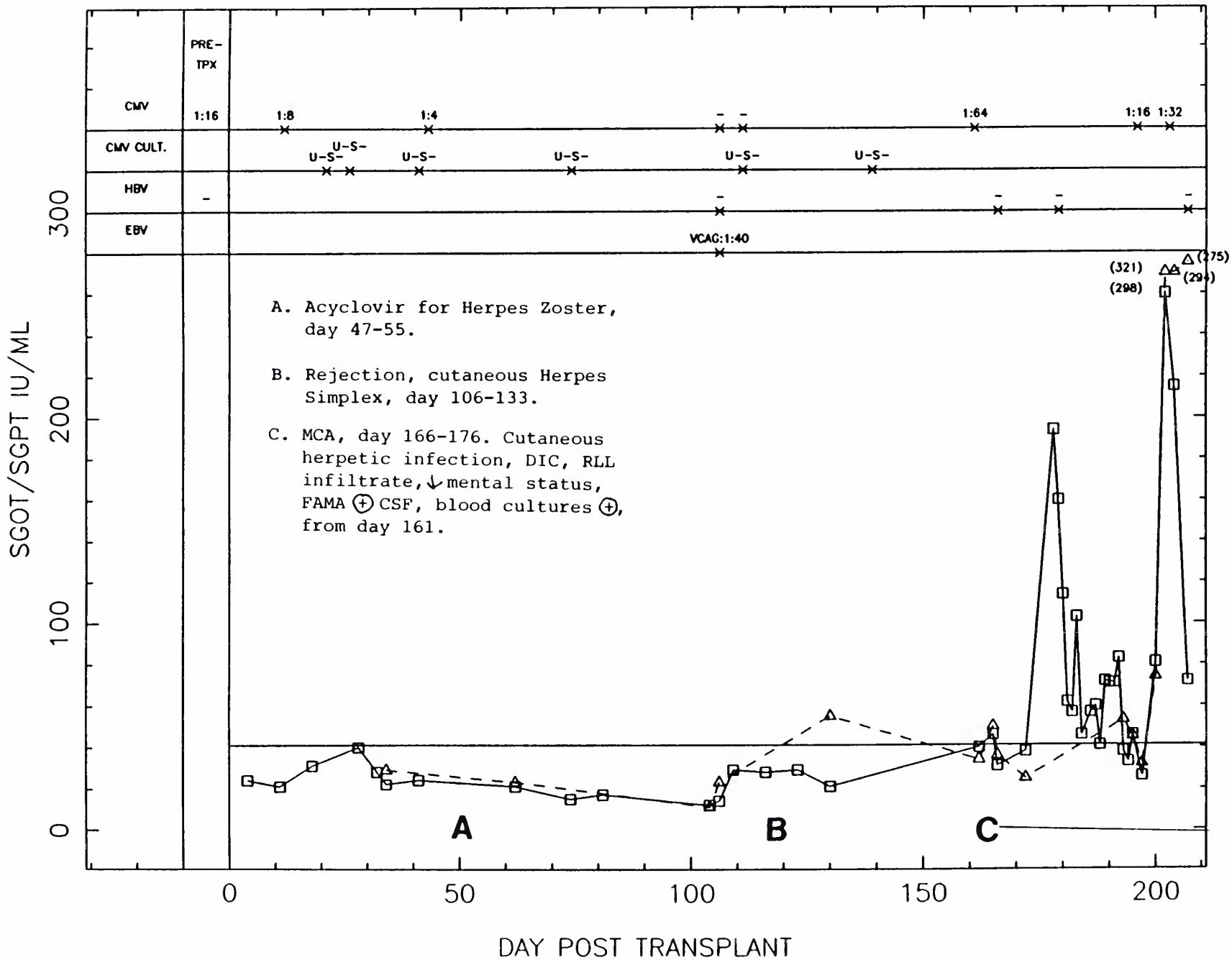
AZATHIOPRINE:PATIENT NUMBER FOUR

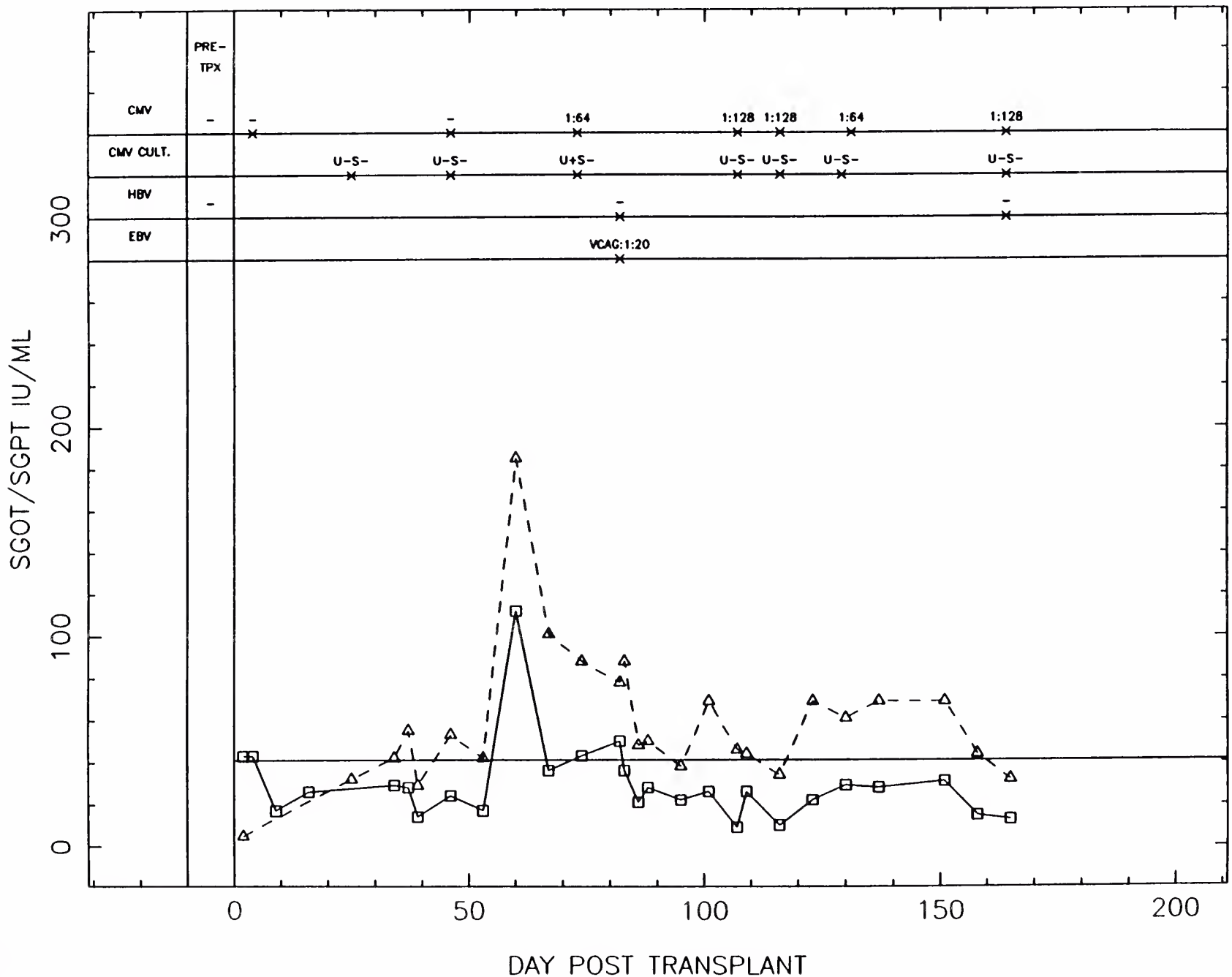


AZATHIOPRINE:PATIENT NUMBER FIVE

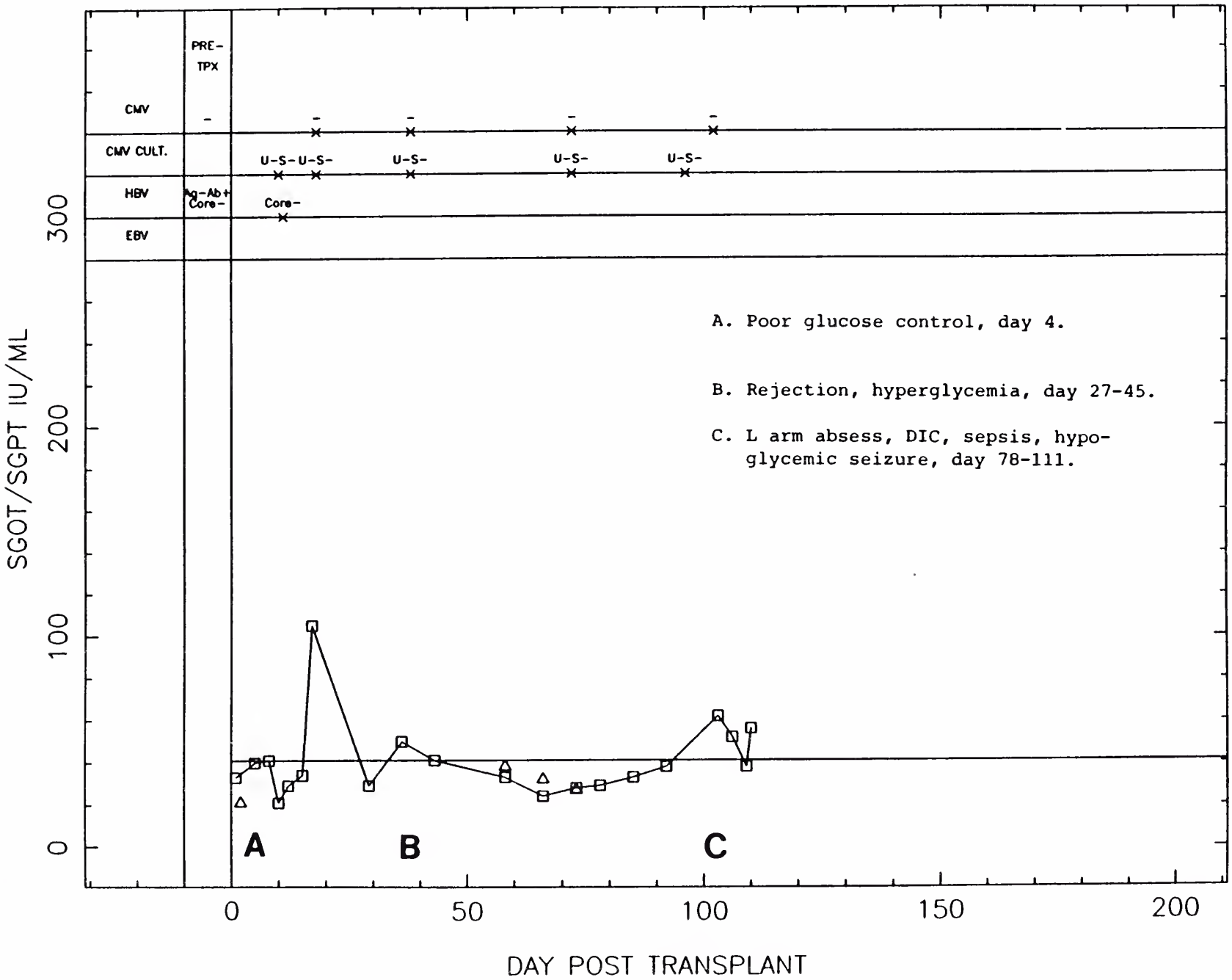


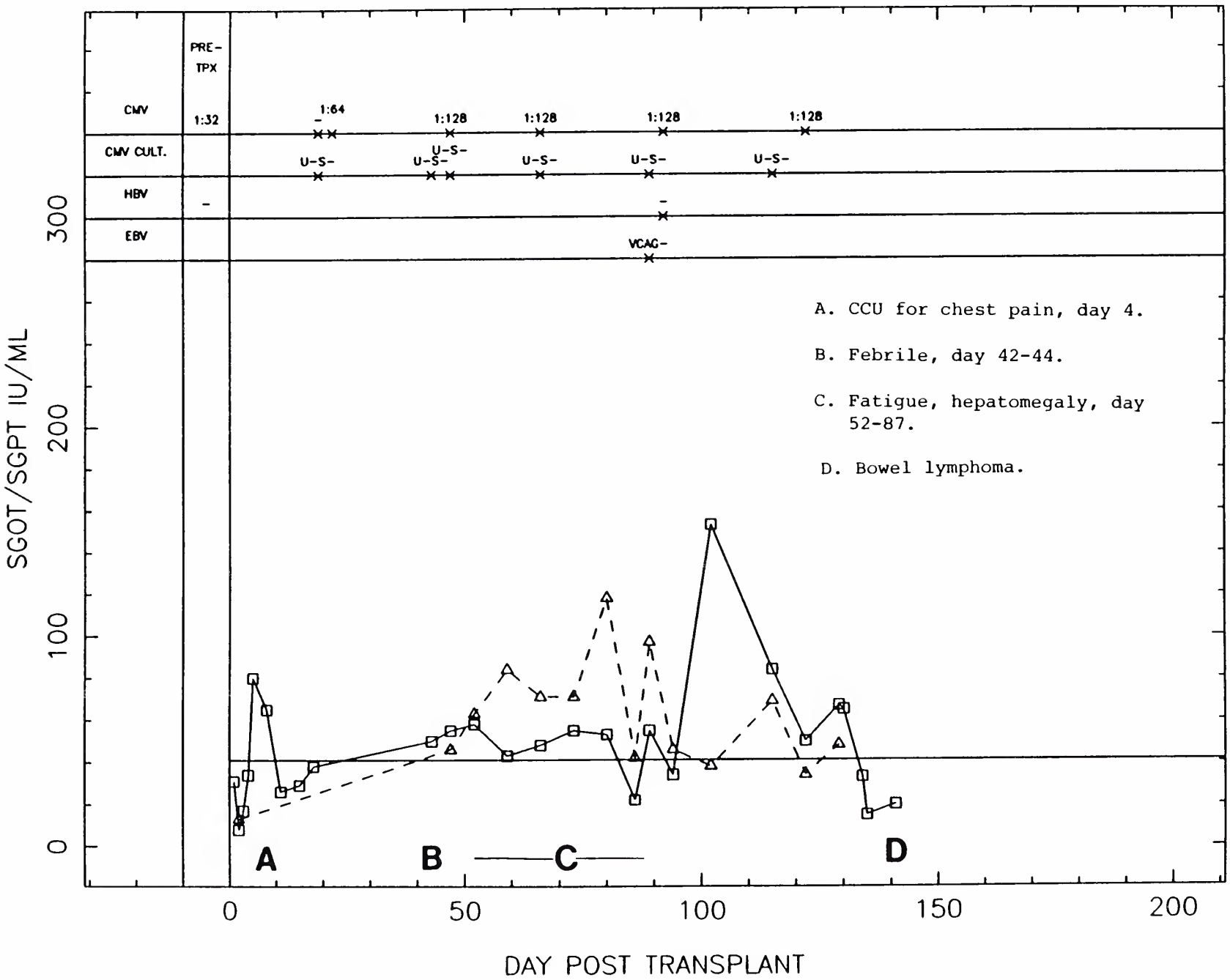
AZATHIOPRINE:PATIENT NUMBER SIX

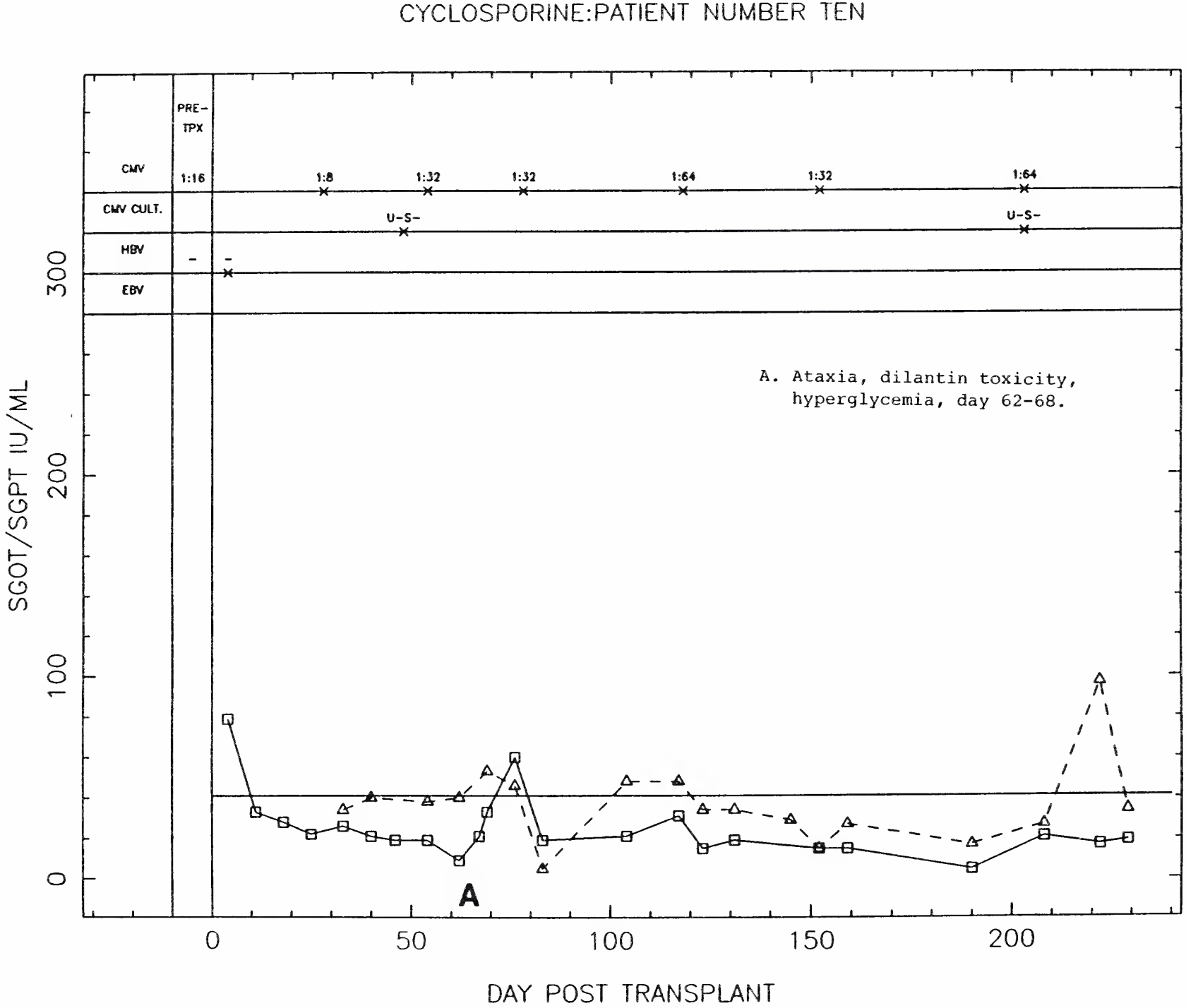


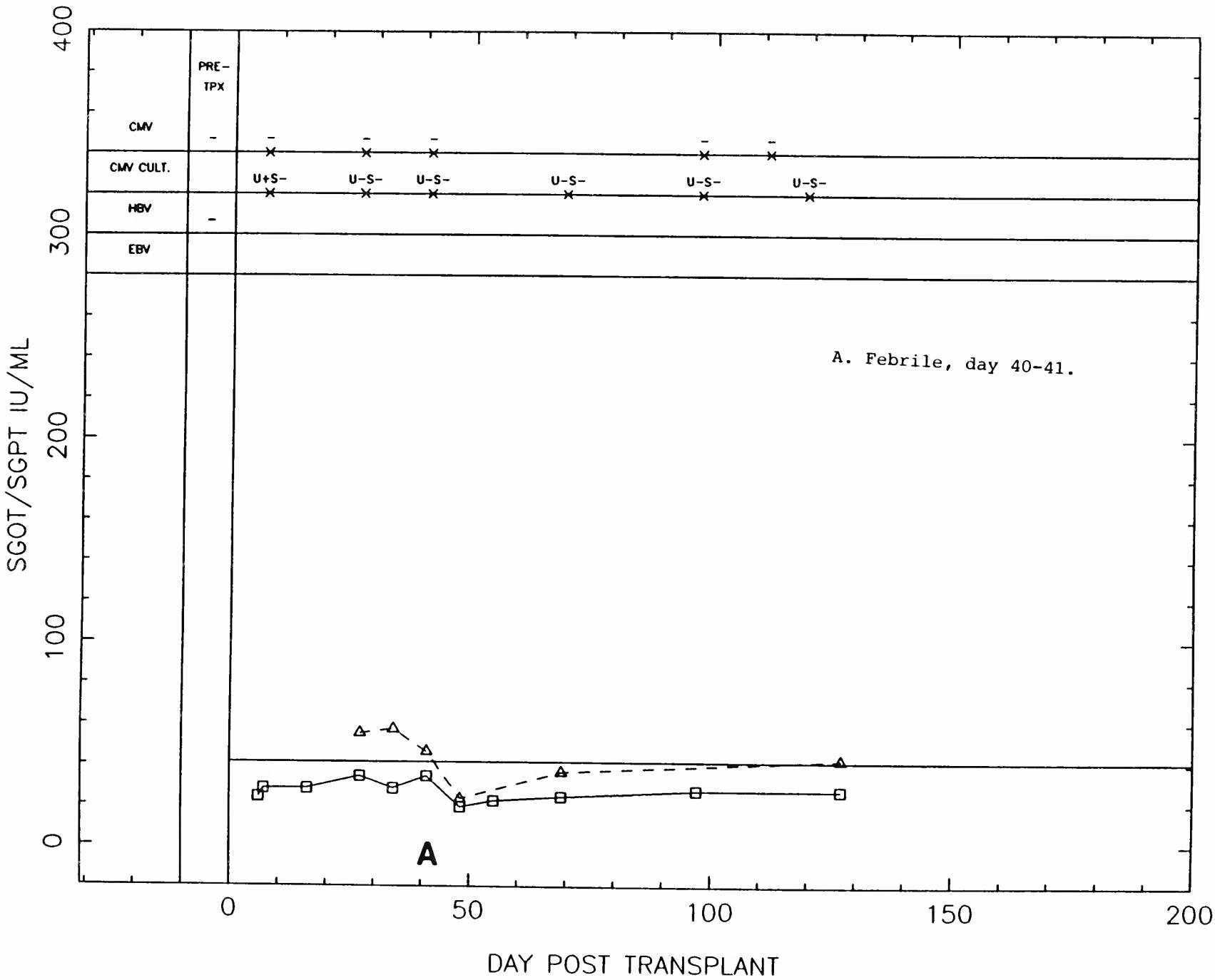

CYCLOSPORINE:PATIENT NUMBER SEVEN
PRE-TPX
CMV
CMV CULT.
HBV
EBV
-
-
-
1:64
1:128
1:128
1:64
1:128
U-S-
U-S-
U+S-
U-S-
U-S-
U-S-
U-S-
-
-
VCAG:1:20
SGOT/SGPT IU/ML
0
100
200
300
0
50
100
150
200
DAY POST TRANSPLANT

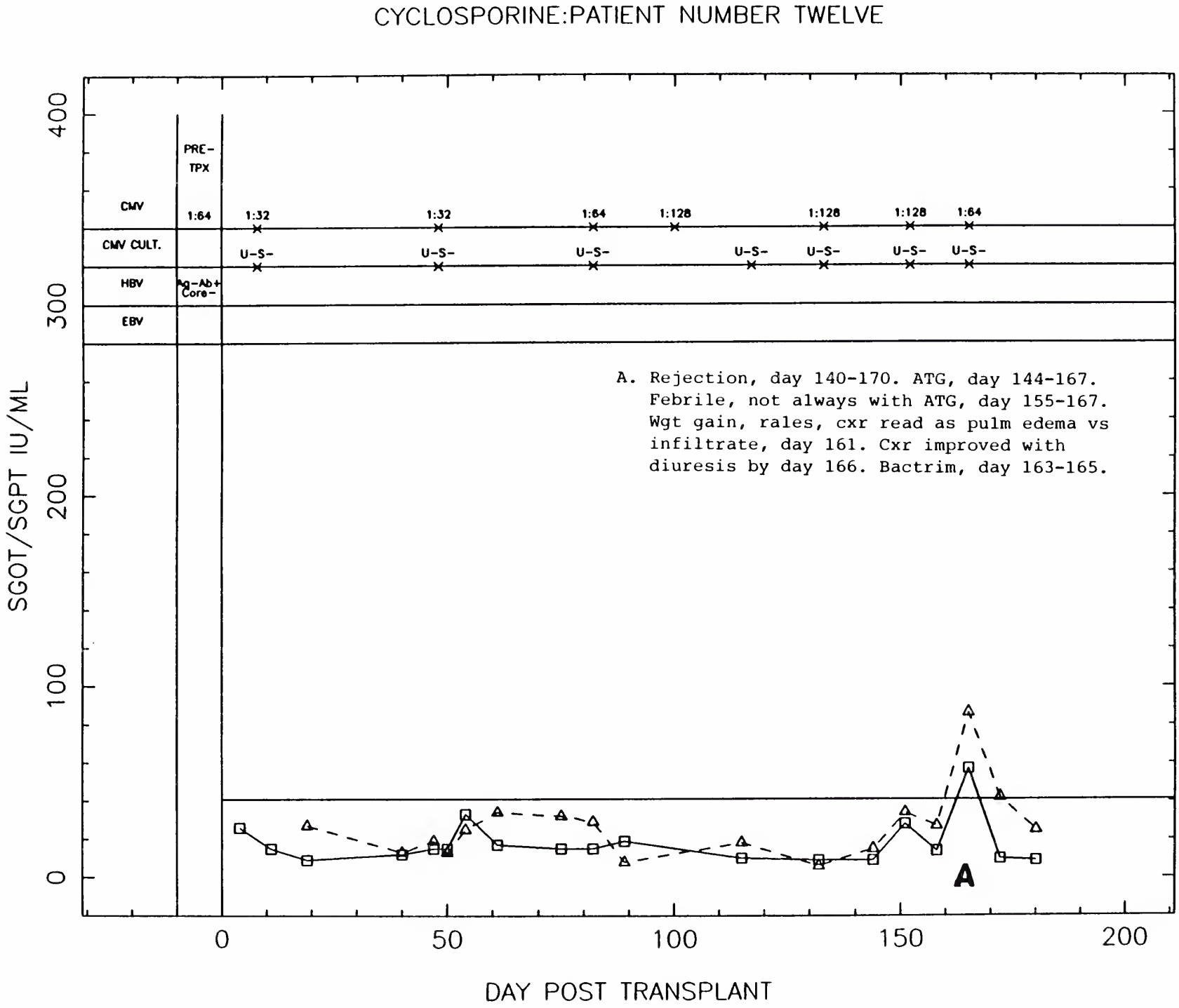
CYCLOSPORINE:PATIENT NUMBER EIGHT



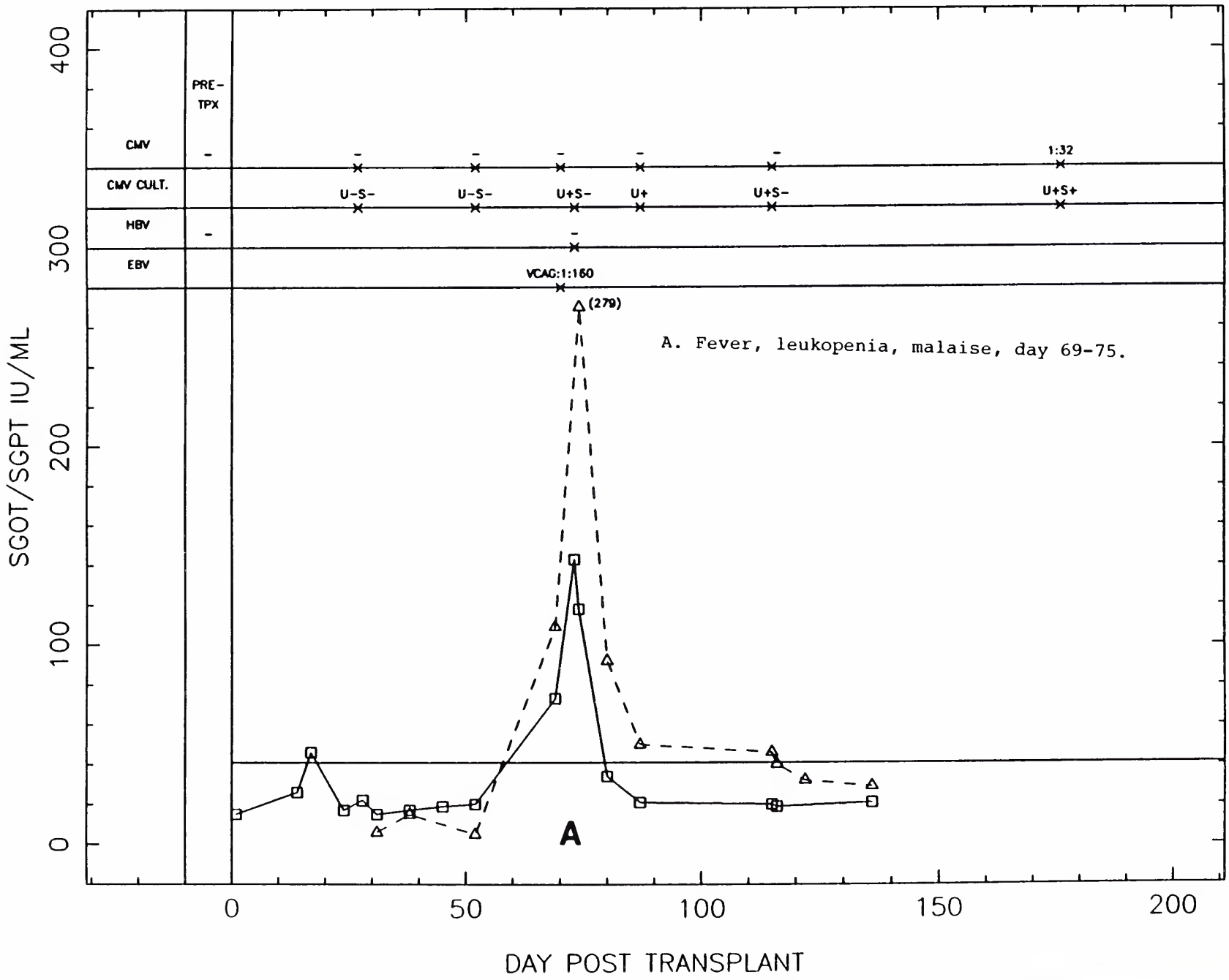

CYCLOSPORINE:PATIENT NUMBER NINE
PRE-TPX
CMV
1:32
1:64
1:128
1:128
1:128
1:128
CMV CULT.
U-S-
HBV
EBV
VCAG-
A. CCU for chest pain, day 4.
B. Febrile, day 42-44.
C. Fatigue, hepatomegaly, day 52-87.
D. Bowel lymphoma.
SGOT/SGPT IU/ML
0
100
200
300
A
B
C
D
0
50
100
150
200
DAY POST TRANSPLANT

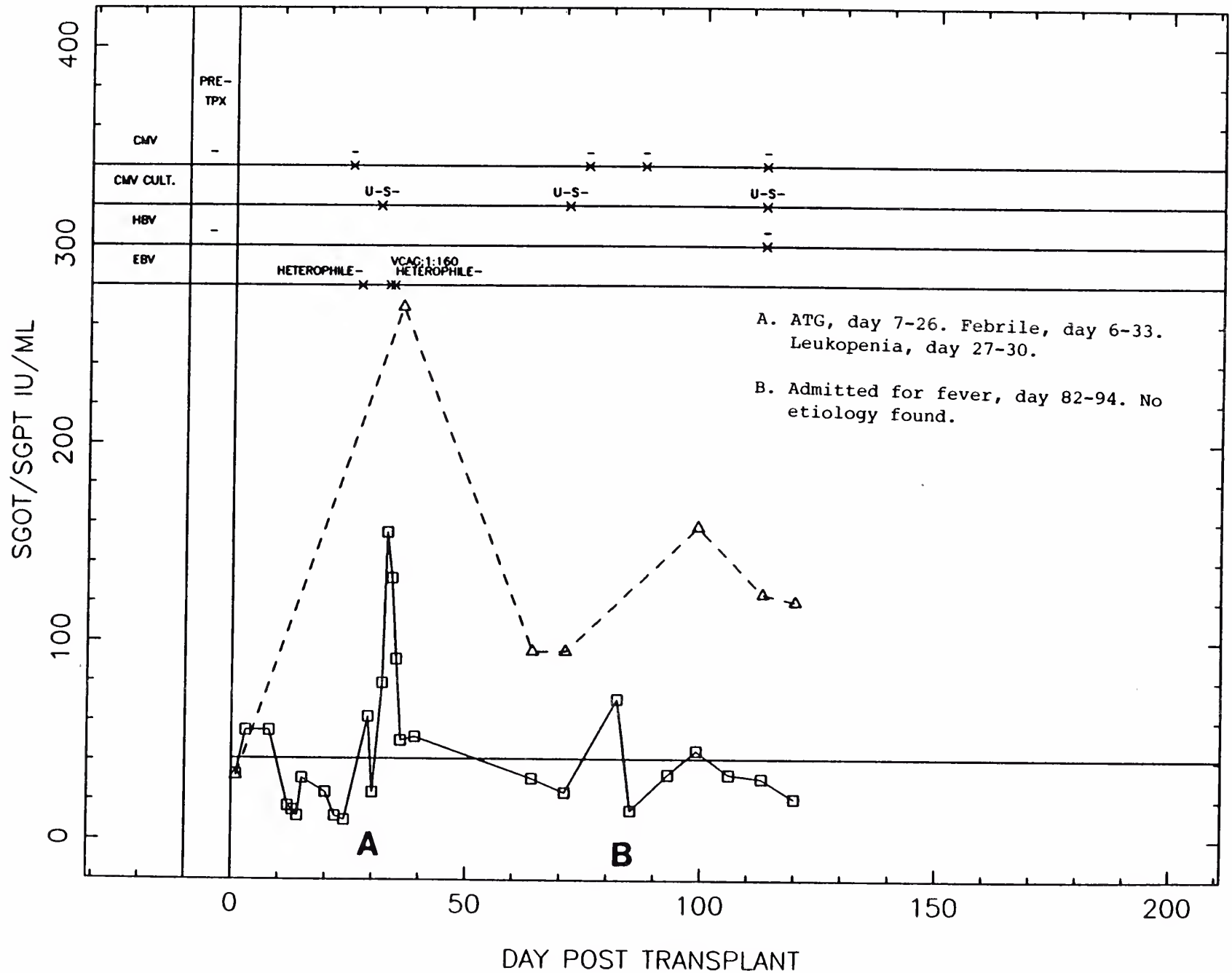

CYCLOSPORINE:PATIENT NUMBER TEN
PRE-TPX
CMV
1:16
1:8
1:32
1:32
1:64
1:32
1:64
CMV CULT.
U-S-
U-S-
HBV
-
-
EBV
A. Ataxia, dilantin toxicity, hyperglycemia, day 62-68.
A
SGOT/SGPT IU/ML
0
100
200
300
0
50
100
150
200
DAY POST TRANSPLANT

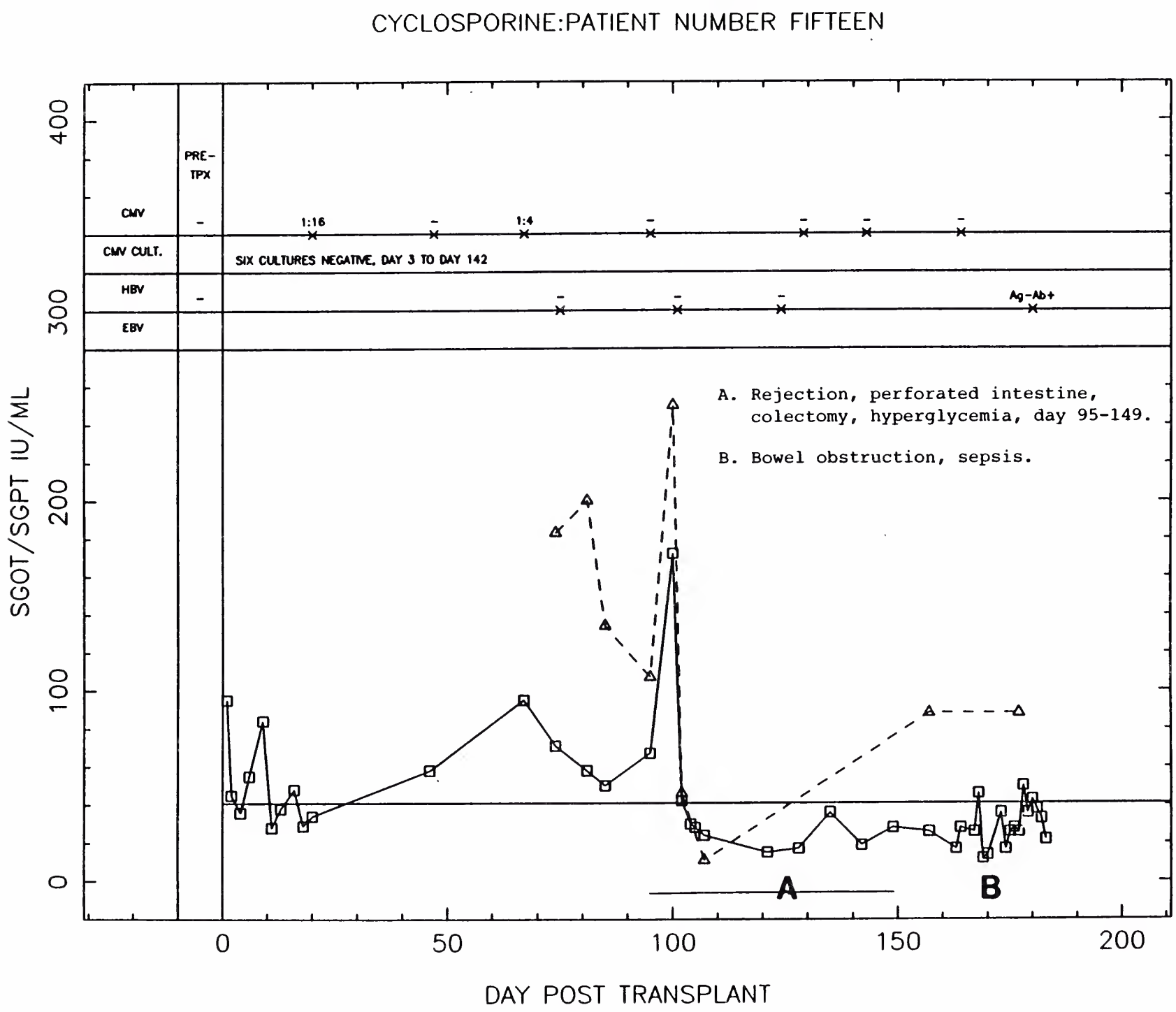

CYCLOSPORINE:PATIENT NUMBER ELEVEN
PRE-TPX
CMV
CMV CULT.
HBV
EBV
U+S-
U-S-
U-S-
U-S-
U-S-
U-S-
A. Febrile, day 40-41.
A
SGOT/SGPT IU/ML
0
100
200
300
400
0
50
100
150
200
DAY POST TRANSPLANT


CYCLOSPORINE:PATIENT NUMBER TWELVE
SGOT/SGPT IU/ML
400
300
200
100
0
PRE-
TPX
CMV
1:64
1:32
1:32
1:64
1:128
1:128
1:128
1:64
CMV CULT.
U-S-
U-S-
U-S-
U-S-
U-S-
U-S-
U-S-
HBV
Ag-Ab+
Core-
EBV
A. Rejection, day 140-170. ATG, day 144-167.
Febrile, not always with ATG, day 155-167.
Wgt gain, rales, cxr read as pulm edema vs
infiltrate, day 161. Cxr improved with
diuresis by day 166. Bactrim, day 163-165.
A
0
50
100
150
200
DAY POST TRANSPLANT

CYCLOSPORINE:PATIENT NUMBER THIRTEEN




CYCLOSPORINE:PATIENT NUMBER FOURTEEN
PRE-TPX
CMV
CMV CULT.
HBV
EBV
U-S-
U-S-
U-S-
HETEROPHILE-
VCAG:1:160
HETEROPHILE-
A. ATG, day 7-26. Febrile, day 6-33. Leukopenia, day 27-30.
B. Admitted for fever, day 82-94. No etiology found.
A
B
SGOT/SGPT IU/ML
0
100
200
300
400
0
50
100
150
200
DAY POST TRANSPLANT


CYCLOSPORINE:PATIENT NUMBER FIFTEEN
PRE-TPX
CMV
CMV CULT.
HBV
EBV
1:16
1:4
SIX CULTURES NEGATIVE, DAY 3 TO DAY 142
Ag-Ab+
A. Rejection, perforated intestine, colectomy, hyperglycemia, day 95-149.
B. Bowel obstruction, sepsis.
A
B
SGOT/SGPT IU/ML
0
100
200
300
400
0
50
100
150
200
DAY POST TRANSPLANT

REFERENCES

1. Rogers AJ, Kahan BD. Mechanism of Action and Clinical Applications of Cyclosporin in Organ Transplantation. Clinics in Immunology and Allergy. 1984; 4:217-258.

2. Kahan BD, Van Buren CT, Flechner SM, Jarowenko M, Yasumura T, Rogers AJ, Yoshimura N, LeGrue S, Drath D, Kerman RH. Clinical and Experimental Studies with Cyclosporine in Renal Transplantation. Surgery. 1985; 97:125-140.

3. Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB. Cyclosporine: A New Immunosuppressive Agent for Organ Transplantation. Annals of Internal Medicine. 1984; 101:667-682.

4. Wood AJ, Lemaire M. Pharmacologic Aspects of Cyclosporine Therapy: Pharmacokinetics. Transplantation Proceedings. 1985; 17 (Suppl. 1):27-32.

5. Maurer G. Metabolism of Cyclosporine. Transplantation Proceedings. 1985; 17 (Suppl. 1):19-26.

6. Cockburn I. Cyclosporine A: A Clinical Evaluation of Drug Interactions. Transplantation Proceedings. 1986; 18 (Suppl. 5):50-55.

7. Steinmuller DR. Usefulness of Cyclosporing Levels One to Six Months Posttransplant. Transplantation Proceedings. 1986; 18 (Suppl. 1):158-164.

8. Wood AJ, Maurer G, Neiderberger W, Beveridge T. Cyclosporine: Pharmacokinetics, Metabolism and Drug Interactions. Transplantation Proceedings. 1983; 15 (Suppl. 1):2409-2412.

9. Keown PA, Stiller CR, Sinclair NR, Carruthers G, Howson W, Stawecki M, McMichael J, Koegler J, McKenzie N, Wall W. The Clinical Relevance of Cyclosporine Blood Levels as Measured by Radioimmunoassay. Transplantation Proceedings. 1983; 15:2438-2441.

10. Holt DW, Marsden JT, Johnston A. Measurement of Cyclosporine: Methodological Problems. Transplantation Proceedings. 1986; 18 (Suppl. 5):101-110.

11. Calne RY, Thiru S, McMaster P, Craddock GN, White DJG, Evans DB, Dunn DC, Pentlow BD, Rolles K. Cyclosporine A in Patients Receiving Renal Allografts from Cadaver Donors. Lancet. 1978; 2:1321-1327.

12. Ryffel B, Hiestand P, Foxwell B, Donatsch P, Boelsterli HJ, Maurer G, Mihatsch MJ. Nephrotoxic and Immunosuppressive Potentials of Cyclosporine Metabolites in Rats. Transplantation Proceedings. 1986; 18 (Suppl. 5):41-45.

13. Robinson WT, Schran HF, Barry EP. Methods to Measure Cyclosporine Levels - High Pressure Liquid Chromatography, Radioimmunoassay, and Correlation. Transplantation Proceedings. 1983; 15:2403-2408.

14. Kahan BD, Van Buren CT, Lin SN, Ono Y, Agostino G, LeGrue SJ, Boileau M, Payne WD, Kerman RH. Immunopharmacological Monitoring of Cyclosporin A-Treated Recipients of Cadaveric Kidney Allografts. Transplantation. 1982; 34:36-45.

15. Burckart G, Starzl T, Williams L, Sanghvi A, Garther C, Venkataramanan R, Zitelli B, Malatack J, Urbach A, Diven W, Ptachinski R, Shaw B, Iwatsuki S. Cyclosporine Monitoring and Pharmacokinetics in Pediatric Liver Transplant Patients. Transplantation Proceedings. 1985; 17:1172-1175.

16. Vine W, Jatlow P, Flye MW. Monitoring of Cyclosporine Concentrations in Blood and Bile After Renal and Hepatic Transplantation. Transplantation Proceedings. 1985; 17:1247-1248.

17. European Multicentre Trial Group. Cyclosporin in Cadaveric Renal Transplantation: One-Year Follow-up of a Multicentre Trial. Lancet. 1983; 2:986-989.

18. The Canadian Multicentre Transplant Study Group. A Randomized Clinical Trial of Cyclosporine in Cadaveric Renal Transplantation. N Engl J Med. 1983; 309:809-815.

19. Najarian JS, Fryd DS, Strand M, Canafax DM, Ascher NL, Payne WD, Simmons RL, Sutherland DER. A Single Institition, Randomized, Prospective Trial of Cyclosporine Versus Azathioprine-Antilymphocyte Globulin for Immunosuppression in Renal Allograft Recipients. Ann Surg. 1985; 201:142-157.

20. Sheil AGR, Hall BM, Tiller DJ, Stephen MS, Harris JP, Duggin GG, Horvath JS, Johnson JR, Rogers JR, Boulas J. Australian Trial of Cyclosporine in Cadaveric Donor Renal Transplantation. Transplantation Proceedings. 1983; 15:2485-2489.

21. Halloran P, Ludwin D, Aprile M, Lien J, White N. Randomized Comparison Between Cyclosporine and Conventional Therapy Plus Minnesota Antilymphocyte Globulin in Cadaveric Renal Transplantation. Transplantation Proceedings. 1983; 15:2513-1516.

22. Canafax DM, Simmons RL, Sutherland DER, Fryd DS, Strand MH, Ascher NL, Payne WD, Najarian JS. Early and Late Effects of Two Immunosuppressive Drug Protocols on Recipients of Renal Allografts: Results of the Minnesota Randomized Trial Comparing Cyclosporine Versus Antilymphocyte Globulin-Azathioprine. Transplantation Proceedings. 1986; 18 (Suppl. 1):192-196.

23. The Canadian Multicentre Transplant Study Group. A Randomized Clinical Trial of Cyclosporine in Cadaveric Renal Transplantation. Analysis at Three Years. N Eng J Med. 1986; 314:1219-1225.

24. Calne RY, Wood AJ. Cyclosporin in Cadaveric Renal Transplantation: 3-Year Follow-up of a European Multicentre Trial. Lancet. 1985; 2:549.

25. Kahan BD, Kerman RH, Wideman CA, Flechner SM, Jarowenko M, Van Buren CT. Impact of Cyclosporine on Renal Transplant Practice at the University of Texas Medical School at Houston. Am J Kid Dis. 1985; 5:288-295.

26. Merion RM, White DJG, Thiru S, Evans DB, Calne RY. Cyclosporine: Five Years' Experience in Cadaveric Renal Transplantation. N Eng J Med. 1984; 310:148-154.

27. Tilney NL, Milford EL, Carpenter CB, Lazarus JM, Strom TB, Kirkman RL. Long-Term Results of Cyclosporine Treatment in Renal Transplantation. Transplantation Proceedings. 1986; 18 (Suppl. 1):179-185.

28. Calne RY, Rolles K, Thiru S, McMaster P, Craddock GN, Aziz S, White DJG, Evans DB, Dunn DC, Henderson RG, Lewis P. Cyclosporin A Initially as the Only Immunosuppressant in 34 Recipients of Cadaveric Organs: 32 Kidneys, 2 Pancreases and 2 Livers. Lancet. 1979; 2:1033-1036.

29. Loertscher R, Thiel G, Harder F, Brunner FP. Persistent Elevation of Alkaline Phosphatase in Cyclosporine-Treated Renal Transplant Recipients. Transplantation. 1983; 36:115-116.

30. Starzl TE, Klintmalm GB, Weil R, Porter KA, Iwatsuki S, Schroter GP, Fernandez-Bueno C, MacHugh N. Cyclosporine A and Steroid Therapy in Sixty-Six Cadaver Kidney Recipients. Surg Gynecol Obstet. 1981; 153:486-494.

31. Starzl TE, Iwatsuki S, Klintmalm G, Schroter GP, Koep LJ, Iwaki Y, Terasaki PI, Porter KA. The Use of Cyclosporine A and Prednisone in Cadaver Kidney Transplantation. Surg Gynecol Obstet. 1980; 151:17-26.

32. Gordon RD, Iwatsuki S, Shaw BW, Starzl TE. Cyclosporine-Steroid Combination Therapy in 84 Cadaveric Renal Transplants. Am J Kid Dis. 1985; 5:307-312.

33. Milford EL, Kirkman RL, Tilney NL, Strom TB, Carpenter CB. Clinical Experience with Cyclosporine and Azathioprine at Brigham and Women's Hospital. Am J Kid Dis. 1985; 5:313-317.

34. Tilney NL, Milford EL, Araujo JL, Strom TB, Carpenter CB, Kirkman RL. Experience with Cyclosporine and Steroids in Clinical Renal Transplantation. Ann Surg. 1984; 200:605-613.

35. European Multicentre Trial Group. Cyclosporin A as Sole Immunosuppressive Agent in Recipients of Kidney Allografts from Cadaver Donors. Lancet. 1982; 2:57-60.

36. Oka T, Ohmori Y, Aikawa I, Ioka J, Kadotani Y, Nomura H, Suzuki S, Hashimoto I. Living Related Kidney Transplants Treated with Cyclosporine. Transplantation Proceedings. 1983; 15:2501-2506.

37. Laupacis A. Complications of Cyclosporine Therapy - A Comparison to Azathioprine. Transplantation Proceedings. 1983; 15:2748-2753.

38. Klintmalm G, Sawe J, von Bahr C, Ringden O, Lundgren G, Wilczek H, Tyden G, Groth CG. Optimal Cyclosporine Plasma Levels Decline with Time of Therapy. Transplantation Proceedings. 1984; 16:1208-1211.

39. Klintmalm G, Sawe J, Ringden O, von Bahr C, Magnusson A. Cyclosporine Plasma Levels in Renal Transplant Patients. Transplantation. 1985; 39:132-137.

40. Loertscher R, Wenk M, Harder F, Brunner F, Follath F, Thiel G. Hyperbilirubinaemia and Cyclosporin A Levels in Renal Transplant Patients. Lancet. 1981; 2:635-636.

41. Laupacis A, Keown PA, Ulan RA, Sinclair NR, Stiller CR. Hyperbilirubinaemia and Cyclosporin A Levels. Lancet. 1981; 2:1426-1427.

42. Klintmalm GB, Iwatsuki S, Starzl TE. Cyclosporin A Hepatotoxicity in 66 Renal Allograft Recipients. Transplantation. 1981; 32:488-489.

43. Keown PA, Stiller CR, Laupacis AL, Howson W, Coles R, Stawecki M, Koegler J, Carruthers G, McKenzie N, Sinclair NR. The Effects and Side-Effects of Cyclosporine: Relationship to Drug Pharmacokinetics. Transplantation Proceedings. 1982; 14:659-661.

44. Rodger RSC, Turney JH, Haines I, Michael J, Adu D, McMaster P. Cyclosporine and Liver Function in Renal Allograft Recipients. Transplantation Proceedings. 1983; 15:2754-2756.

45. Rodger S, Turney JH, Haynes I, McMaster P, Michael J, Adu D. Normal Liver Function in Renal Allograft Recipients Treated with Cyclosporine. Transplantation. 1983; 36:451-452.

46. Maddux MS, Frigo LC, Veremis SA, Pollak R, Mozes MF. Cyclosporine Hepatotoxicity Following Renal Transplantation. Clin Pharmacol Therap. 1986; 39:208.

47. Lorber MI, Van Buren CT, Flechner SM, Williams C, Kahan BD. Hepatobiliary and Pancreatic Complications of Cyclosporine

Therapy in 466 Renal Transplant Recipients. Transplantation. 1987; 43;35-40.

48. Schade RR, Guglielmi A, Van Thiel DH, Thompson ME, Warty V, Griffith B, Sanghvi A, Bahnson H, Hardesty R. Cholestasis in Heart Transplant Recipients Treated with Cyclosporine. Transplantation Proceedings. 1983; 15:2757-2760.

49. Atkinson K, Biggs J, Dodds A, Concannon A. Cyclosporine-Associated Hepatotoxicity After Allogeneic Marrow Transplantation in Man: Differentiation From Other Causes of Posttransplant Liver Disease. Transplantation Proceedings. 1983; 15:2761-2767.

50. Sopko J, Anuras S. Liver Disease in Renal Transplant Recipients. Am J Med. 1978; 64:139-146.

51. LaQuaglia MP, Tolkoff-Rubin NE, Dienstag JL, Cosimi B, Herrin JT, Kelly M, Rubin RH. Impact of Hepatitis on Renal Transplantation. Transplantation. 1981; 32:504-507.

52. Kirkman RL, Strom TB, Weir MR, Tilney NL. Late Mortality and Morbidity in Recipients of Long-Term Renal Allografts. Transplantation. 1982; 34:347-351.

53. Weir MR, Kirkman RL, Strom TB, Tilney NL. Liver Disease in Recipients of Long-Functioning Renal Allografts. Kidney International. 1985; 28:839-844.

54. Rubin RH, Wolfson JS, Cosimi AB, Tolkoff-Rubin NE. Infection in the Renal Transplant Recipient. Am J Med. 1981; 70:405-411.

55. Glenn J. Cytomegalovirus Infections Following Renal Transplantation. Reviews of Infectious Diseases. 1981; 3:1151-1177.

56. Peterson PK, Balfour HH, Marker SC, Fryd DS, Howard RJ, Simmons RL. Cytomegalovirus Disease in Renal Allograft Recipients: A Prospective Study of the Clinical Features, Risk Factors and Impact on Renal Transplantation. Medicine. 1980; 59:283-299.

57. Chatterjee SN, Jordan GW. Prospective Study of the Prevalence and Symptomatology of Cytomegalovirus Infection in Renal Transplant Recipients. Transplantation. 1979; 28:457-460.

58. Whelchel JD, Pass RF, Diethelm AG, Whitley RJ, Alford CA. Effect of Primary and Recurrent Cytomegalovirus Infections upon Graft and Patient Survival After Renal Transplantation. Transplantation. 1979; 28:443-446.

59. Fryd DS, Peterson PK, Ferguson RM, Simmons RL, Balfour HH, Najarian JS. Cytomegalovirus as a Risk Factor in Renal Transplantation. Transplantation. 1980; 30:436-439.

60. Marker SC, Howard RJ, Simmons RL, Kalis JM, Connelly DP, Najarian JS, Balfour HH. Cytomegalovirus Infection: A Quantitative Prospective Study of Three-Hundred Twenty Consecutive Renal Transplants. Surgery. 1981; 89:660-671.

61. Warrell MJ, Chinn I, Morris PJ, Tobin JO. The Effects of Viral Infections on Renal Transplants and Their Recipients. Quarterly Journal of Medicine. 1980; 94:219-231.

62. Ware AJ, Luby JP, Hollinger B, Eigenbrodt EH, Cuthbert JA, Atkins CR. Shorey J, Hull AR, Combes B. Etiology of Liver Disease in Renal-Transplant Patients. Ann Int Med. 1979; 91:364-371.

63. Mozes MF, Ascher NL, Balfour HH, Simmons RL, Najarian JS. Jaundice After Renal Allotransplantation. Ann Surg. 1978; 188:783-790.

64. Luby JP, Burnett W, Hull AR, Ware AJ, Shorey JW, Peters PC. Relationship Between Cytomegalovirus and Hepatic Function Abnormalities in the Period After Renal Transplant. The Journal of Infectious Diseases. 1974; 129:511-518.

65. Ware AJ, Luby JP, Eigenbrodt EH, Long DL, Hull AR. Spectrum of Liver Disease in Renal Transplant Recipients. Gastroenterology. 1975; 68:755-763.

66. Anuras S, Piros J, Bonney WW, Forker EL, Colville DS, Corry RJ. Liver Disease in Renal Transplant Recipients. Arch Intern Med. 1977; 137:42-48.

67. Marker SC, Ascher NL, Kalis JM, Simmons RL, Najarian JS, Balfour HH. Epstein-Barr Virus Antibody Responses and Clinical Illness in Renal Transplant Recipients. Surgery. 1979; 85:433-440.

68. Cheeseman SH, Henle W, Rubin RH, Tolkoff-Rubin NE, Cosimi B, Cantell K, Winkle S, Herrin JT, Black PH, Russell PS, Hirsch MS. Epstein-Barr Virus Infection in Renal Transplant Recipients. Ann Int Med. 1980; 93:39-42.

69. Berne TV, Chatterjee SN, Craig JR, Payne JE. Hepatic Dysfunction in Recipients of Renal Allografts. Surg, Gynecol Obstet. 1975; 141:171-175.

70. Degos F, Degott C, Bedrossian J, Camilieri JP, Barbanel C, Duboust A, Rueff B, Benhamou JP, Kreis H. Is Renal Transplantation Involved in Post-Transplantation Liver Disease? Transplantation. 1980; 29:100-102.

71. Ireland P, Rashid A, von Lichtenberg F, Cavallo T, Merrill JP. Liver Disease in Kidney Transplant Patients Receiving Azathioprine. Arch Intern Med. 1973; 132:29-37.

72. Millard PR, Herbertson BM, Evans DB, Calne RY. Azathioprine Hepatotoxicity in Renal Transplantation. Transplantation. 1973; 16:527-530.

73. Cello JP, Sleisenger MH. The Liver in Systemic Conditions. In: Zakim D, Boyer TD, eds. Hepatology A Textbook of Liver Disease. Philadelphia: W.B. Saunders and Co.; 1982:1221-1249.

74. Balfour HH, Slade MS, Kalis JM, Howard RJ, Simmons RL, Najarian JS. Viral Infections in Renal Transplant Donors and Their Recipients: A Prospective Study. Surgery. 1977; 81:487-492.

75. Bia MJ, Andiman W, Gaudio K, Kliger A, Siegel N, Smith D, Flye W. The Effect of Treatment with Cyclosporine Versus Azathioprine on Incidence and Severity of Cytomegalo Virus Infection Posttransplantation. Transplantation. 1985; 40:611-614.

76. Ozguc L, Duncan LJP. Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminase Levels in Diabetes Mellitus. Clin Chim Acta. 1963; 8:586-590.

77. Bia MJ, Flye WM. Immunoblastic Lymphoma in a Cyclosporine-Treated Renal Transplant Recipient. Transplantation. 1985; 39:673-675.

78. Maddrey WC. Drug and Chemical-Induced Hepatic Injury. In: Haubrich WS, Kalser MH, Roth JLA, Schaffner F, eds. Bockus Gastroenterology. Philadelphia: W.B. Saunders and Co.; 1985:2922-2956.

79. Hoffnagle JH. Acute Viral Hepatitis: Clinical Features, Laboratory Findings, and Treatment. In: Haubrich WS, Kalser MH, Roth JLA, Schaffner F, eds. Bockus Gastroenterology. Philadelphia: W.B. Saunders and Co.; 1985:2856-2901.

80. Rotolo FS, Branum GD, Bowers BA, Meyers WC. Effect of Cyclosporine on Bile Secretions in Rats. Am J of Surgery. 1986; 151:35-40.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS	DATE
John Mott 169 Dwight St	3/1/89